P. ENT COOPERATION TREA

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 July 2000 (20.07.00)	
International application No. PCT/GB99/04399	**Applicant's or agent's file reference** SJK/BP5827712
International filing date (day/month/year) 23 December 1999 (23.12.99)	**Priority date** (day/month/year) 24 December 1998 (24.12.98)
Applicant SCHOFIELD, Julian et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 June 2000 (08.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer Pascal Piriou Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

GB9904399

PCT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
KIDDLE, Simon J. et al.
Mewburn Ellis
York House
London WC2B 6HP
UNITED KINGDOM

RECEIVED
18 SEP 2000

Applicant's or agent's file reference SJK/BP5827712	Date of mailing *(day/month/year)* 14/09/2000
International application No. PCT/GB 99/04399	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant UNIVERSITY COLLEGE LONDON et al.	International filing date *(day/month/year)* 23/12/1999

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Doreen Golze

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
SJK/BP5827712		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/04399	23/12/1999	24/12/1998
Applicant		
UNIVERSITY COLLEGE LONDON et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readble form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

HUMAN GLYCOSYLPHOSPHATIDYLINOSITOL SPECIFIC PHOSPHOLIPASE D VARIANTS AND USES THEREOF

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

☐ None of the figures.

☐ as suggested by the applicant.

☒ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 99/04399

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/55 C12N9/16 C12Q1/34 G01N33/48 A61K38/46
A61K48/00 A61P1/18 A61P1/16 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q G01N A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAGUIRE, G.A. & GOSSNER, A.: "Glycosyl phosphatidyl inositol phospholipase D activity in human serum" ANNALS OF CLINICAL BIOCHEMISTRY, vol. 32, no. 1, January 1995 (1995-01), pages 74-78, XP000864653 abstract page 75, column 1, line 1 - line 39 page 76; figures 3A,C page 77, column 1, line 2 -page 78, column 1, line 13 --- -/--	4,13-16, 28,30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2000	14. 9. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VICENT, D. ET AL.: "Alterations in Skeletal Muscle Gene Expression" DIABETES, vol. 47, no. 9, September 1998 (1998-09), pages 1451-1458, XP000864657 abstract page 1454, column 1, line 9 - line 37 page 1457, column 1, line 30 - line 54 ---	4,7-12
A	EP 0 477 739 A (F. HOFFMANN-LA ROCHE AG) 1 April 1992 (1992-04-01) cited in the application abstract page 4, line 14 -page 5, line 8 page 7, line 52 -page 11, line 30; tables 1,2 page 20 -page 21; claims 1,2,5-17 page 31 -page 37; figures 9,10 ---	1-31
Y		36-45
A	SCALLON, B.J. ET AL.: "Primary Structure and Fucntional Acitvity of a Phosphatidylinositol-Glycan-Specific Phospholipase D" SCIENCE, vol. 252, no. 5004, 19 April 1991 (1991-04-19), pages 446-448, XP000293055 cited in the application abstract page 446, column 2, line 3 -page 447, column 1, line 2 page 447, column 1, line 26 - line 30 page 447, column 1, line 36 -column 2, line 6; figure 3 ---	1-31
Y		36-45
P,X	WO 99 47565 A (RADEMACHER GROUP LIMITED) 23 September 1999 (1999-09-23) abstract page 23, line 16 -page 24, line 7 page 24, line 18 - line 29 page 31 -page 35; examples 3,4 page 43-46; claims 1-5,10-14,16-19,22 page 48 -page 50; figures 2-4 --- -/--	28,29,31

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TSANG, T.C. ET AL.: "Isolation and expression of two human glycosylphosphatidylinositol phospholipase D (GPI-PLD) cDNAs" FASEB JOURNAL, vol. 6, April 1992 (1992-04), page A1922 XP000907489 cited in the application abstract no.: 5707 the whole document	1-31, 36-45
A	-& EMBL Database, Heidelberg, FRG accession number L11702 07 September 1993 TSANG, T.C. ET AL: "Human phospholipase D mRNA, complete cds" XP002141248 cited in the application the whole document	1-31, 36-45
A	HOENER, M.C. & BRODBECK, U.: "Phosphatidylinositol-glycan-specific phospholipase D is an amphiphilic glycoprotein that in serum is associated with high-density lipoproteins" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 206, no. 3, June 1992 (1992-06), pages 747-757, XP000913778 cited in the application abstract page 750, column 2, line 13 -page 754, column 2, line 21; figures 2-6; tables 1,2	1-31, 36-45
A	HUANG, L.C ET AL.: "Chiroinositol Deficiency and Insulin Resistence. III. Acute Glycogenic and Hypoglycemic Effects of Two Inositol Phosphoglycan Insulin Mediators in Normal and Streptozotocin-Diabetic Rats in Vivo" ENDOCRINOLOGY, vol. 132, no. 2, January 1993 (1993-01), pages 652-657, XP002050432 the whole document	1-31, 36-45

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/04399

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0477739 A	01-04-1992	JP 5076357 A	30-03-1993
		US 5418147 A	23-05-1995
WO 9947565 A	23-09-1999	AU 2946799 A	11-10-1999

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/04399

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-31, and 36-45 completely

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-31 and 36-45 completely

A glycosylphosphatidylinositol specific phospholipase D (GPI-PLD) and a nucleic acid encoding GPI-PLD for the use in a method of medical treatment; the use of GPI-PLD or a nucleic acid encoding GPI-PLD for the preparation of a medicament for the treatment of diabetes/diabetic complications, liver dysfunction/disorders involving pancreatectomies and a condition mediated by a product of an infectious organism being capable of inhibiting GPI-PLD; the use of the presence or amount of GPI-PLD in a sample derived from a patient in diagnosis; a diagnostic method for diabetes/diabetic complications, liver dysfunction/disorders involving pancreatectomies and a condition mediated by a product of an infectious organism being capable of inhibiting GPI-PLD comprising the determination of the amount of GPI-PLD or a product of GPI-PLD action in a sample derived from a patient; a cell line transformed with a nucleic acid encoding GPI-PLD; the use of said cell line for the preparation of said medicament; a pharmaceutical composition comprising GPI-PLD or a nucleic acid encoding GPI-PLD; a GPI-PLD variant differing in amino acid sequence at positions 689-692 of human wild-type GPI-PLD and a nucleic acid encoding said GPI-PLD variant for the use in a method of medical treatment; an expression vector comprising said nucleic acid encoding said GPI-PLD variant; a host cell transformed with said nucleic acid encoding said GPI-PLD variant; a method of producing said GPI-PLD variant.

2. Claims: 32-35 partially

An isolated human GPI-PLD protein corresponding to clone a1 having an amino acid sequence as shown in Figure 3 and an isolated nucleic acid sequence encoding said GPI-PLD protein as shown in Figure 4; an expression vector comprising said nucleic acid sequence; an isolated nucleic acid sequence encoding a GPI-PLD protein having greater than 90% identity with the nucleic acid sequence as shown in Figure 4.

3. Claims: 32-35 partially

An isolated human GPI-PLD protein corresponding to clone b2 having an amino acid sequence as shown in Figure 3 and an isolated nucleic acid sequence encoding said GPI-PLD protein as shown in Figure 5; an expression vector comprising said nucleic acid sequence; an isolated nucleic acid sequence encoding a GPI-PLD protein having greater than 90% identity with the nucleic acid sequence as shown in Figure 5.

4. Claims: 32-35 partially

An isolated human GPI-PLD protein corresponding to clone d3

having an amino acid sequence as shown in Figure 3 and an isolated nucleic acid sequence encoding said GPI-PLD protein as shown in Figure 6; an expression vector comprising said nucleic acid sequence; an isolated nucleic acid sequence encoding a GPI-PLD protein having greater than 90% identity with the nucleic acid sequence as shown in Figure 6.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

KIDDLE, Simon J. et al.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	21.03.2001
Applicant's or agent's file reference SJK/BP5827712	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04399	23/12/1999	24/12/1998

Applicant
UNIVERSITY COLLEGE LONDON et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Büchler, S Tel.+49 89 2399-8090

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SJK/BP5827712		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04399	23/12/1999	24/12/1998
International Patent Classification (IPC) or national classification and IPC		
C12N15/55		
Applicant		
UNIVERSITY COLLEGE LONDON et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/06/2000	21.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Page, M Telephone No. +49 89 2399 7322

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04399

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:

Description, pages:

1-46 as originally filed

Claims, No.:

1-46 as received on 28/02/2001 with letter of 28/02/2001

Drawings, sheets:

1/20-20/20 as originally filed

Sequence listing part of the description, pages:

1-57 (SEQ ID NOs. 1-30), filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 33-36.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 33-36.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-32, 40, 42
	No:	Claims 37-39, 41, 43-46

Inventive step (IS)	Yes:	Claims	1-32, 40, 42
	No:	Claims	37-39, 41, 43-46
Industrial applicability (IA)	Yes:	Claims	1-32, 37-46
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The application concerns the provision of polynucleotides and polypeptides corresponding to glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) for therapeutic application, methods for diagnosing conditions associated with altered GPI-PLD levels and variant polypeptide and polynucleotide sequences. Several GPI-PLD variants are known in the art, but they have not been disclosed as being suitable for therapeutic use.

Re Item II
Priority

After considering the priority documents, the document cited "P, X" in the search report is not considered relevant for the examination of novelty and inventive step.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The subject matter of **claims 33-36** was not examined due to the non-establishment of a search report for these claims. See International Search Report for details.

Re Item IV
Lack of Unity of Invention

The application concerns the provision of GPI-PLD polypeptides and polynucleotides for use in medical treatment, the provision of supposedly novel GPI-PLD variants (not explicitly for use in medical treatment) and diagnostic methods associated with the polypeptides. Multiple (variant) polynucleotide and polypeptide sequences for GPI-PLD are known in the art (D2 Figs. 9 and 11), as are diagnostic methods for their quantification (D1 page 76 GPI-PLD activity activities in different patient groups). Therefore, the three inventive concepts are not linked to form a common underlying inventive concept as it is considered that there is no special technical feature present. The application does not comply with the requirements for unity of invention (Article 34(3) and Rules 13 and 68 PCT) and the subject matter of the application is therefore

considered to relate not to one, but to 3 separate inventions as follows:

Invention I Claims 1-4, 7-27, 40 and 42: GPI-PLD polypeptides and polynucleotides for use in medical treatment.

Invention II Claims 5, 6 and 28-32: Methods of diagnosis, the methods comprising the determination of GPI-PLD activity in a sample.

Invention III Claims 37-39, 41 and 43-46: Variant GPI-PLD polypeptides and polynucleotides.

N.B. The change in dependency of claims 43-46 has led to the reassessment of the said claims, not only with regard to which invention they belong to, but also with regard to novelty and inventive step.

N.B. the use of the term "invention" here in no way implies recognition of an inventive step for the subject-matter.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

D1: MAGUIRE, G.A. & GOSSNER, A.: 'Glycosyl phosphatidyl inositol phospholipase D activity in human serum' ANNALS OF CLINICAL BIOCHEMISTRY, vol. 32, no. 1, January 1995 (1995-01), pages 74-78, XP000864653

D2: EP-A-0 477 739 (F. HOFFMANN-LA ROCHE AG) 1 April 1992 (1992-04-01) cited in the application

D3: SCALLON, B.J. ET AL.: 'Primary Structure and Functional Activity of a Phosphatidylinositol-Glycan-Specific Phospholipase D' SCIENCE, vol. 252, no. 5004, 19 April 1991 (1991-04-19), pages 446-448, XP000293055 cited in the application

D4: HOENER, M.C. & BRODBECK, U.: 'Phosphatidylinositol-glycan-specific

phospholipase D is an amphiphilic glycoprotein that in serum is associated with high-density lipoproteins' EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 206, no. 3, June 1992 (1992-06), pages 747-757, XP000913778 cited in the application

D5: HUANG, L.C ET AL.: 'Chiroinositol Deficiency and Insulin Resistence. III. Acute Glycogenic and Hypoglycemic Effects of Two Inositol Phosphoglycan Insulin Mediators in Normal and Streptozotocin-Diabetic Rats in Vivo' ENDOCRINOLOGY, vol. 132, no. 2, January 1993 (1993-01), pages 652-657, XP002050432

2) **Novelty - Art.33(1) and (2) PCT**:

Invention I

Claims 1-4, 7-27, 40 and 42 appear to be novel in light of the cited prior art. GPI-PLD for use in medical treatment has not been previously disclosed.

Invention II

Claims 5, 6 and 28-30 appear to be novel in light of the cited prior art. Although D1 discloses the use of an assay for determining the activity of GPI-PLD in human serum and correlates this activity to pathologies of the liver characterised by reduced GPI-PLD levels (D1 page 76 GPI- PLD activity activities in different patient groups), no mention is made of diabetic disorders, pancreatectomies or conditions mediated by the product of infectious organisms that inhibit GPI-PLD.

Claims 31 and 32 also appears to be novel in light of the cited prior art. The technical features of the assay provided in claim 31 are not disclosed in D1.

Invention III

Claims 37-39, 41 and 43-46 cannot be acknowledged as being novel as no sequence is defined in which the provided changes are to be found. The claims could thus apply to any GPI-PLD polypeptide sequence and thus lack novelty in light of e.g. D1 and D2, which disclose further GPI-PLD sequences.

3) **Inventive Step - Art.33(1) and (3) PCT**:

The following comments on inventive step are confined to subject matter which could be acknowledged as being novel, or for which novelty could potentially be restored as outlined supra.

Invention I

The closest prior art is D2, which provides the polynucleotide and polypeptide sequences of bovine GPI-PLD and human liver and pancreas GPI-PLD (D2 Figs. 5, 9 and 11).

In light of the prior art, the technical problem can be regarded as the provision of GPI-PLD for medical use.

The technical problem is solved by the subject matter of claims 1-4, 7-27, 40 and 42, which provide GPI-PLD for therapeutic use.

In light of the cited prior art, there does not appear to be any motivation to prepare GPI-PLD for therapeutic purposes. Although it is known that one of the products of this enzyme, namely inositol phosphoglycan, mediates insulin action (D5 page 656 left-hand column paragraph 2), there does not appear to be any motivation to increase the level of this molecule through treatment with GPI-PLD.

Claims 1-4, 7-27, 40 and 42 therefore appear to demonstrate inventive step in light of the cited prior art.

Invention II

The closest prior art is D1, which provides a diagnostic assay for the detection of GPI-PLD in biological samples (D1 page 76 GPI-PLD activity activities in different patient groups).

In light of the prior art, the technical problem can be regarded as the provision of methods for diagnosing specific conditions in which GPI-PLD is inhibited or depleted by determining the biological activity of GPI-PLD.

The technical problem is solved by the subject matter of claims 5, 6 and 28-32, which provide an association (immuno-type) assay for GPI-PLD or for a product of GPI-PLD action, such as IPG or acyl-IPG.

In light of the cited prior art, **claims 5, 6 and 28-30** appear to be inventive. The prior art does not disclose the diagnosis of the listed conditions using GPI-PLD concentrations or activities.

Claims 31 and 32 also appears to be inventive in light of the cited prior art: Other assays provided by the art rely on the cleavage of enzymes from insoluble supports by GPI- PLD and the subsequent measurement of enzyme activity (e.g. D1 page 75 Assay for GPI-PLD activity in human serum). The assay provided in claim 31, however, relies on the immobilisation of the enzyme on a solid support using a GPI-PLD binding protein and quantifying unoccupied binding sites.

Invention III

The closest prior art is D2, which provides 3 GPI-PLD polypeptide and polynucleotide sequence pairs (D2 Figs. 5, 9 and 11).

In light of the prior art, the technical problem can be regarded as the provision of further variant GPI-PLD polypeptide and polynucleotide sequences.

The technical problem is solved by the subject matter of claims 37-39, 41 and 43-46.

Even if novelty had been restored to claims 36-38 and 40 by defining the sequence for which protection is sought, it cannot be seen how the subject matter of **claims 37-39, 41 and 43-46** could be regarded as inventive. In the absence of any form of functional statement or support in the form of specific examples of these variants, it is not possible to acknowledge inventive step.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 47565	23/09/1999	18/03/1999	18/03/1998 21/05/1998

Document relevant to claims 28, 29 and 31.

Re Item VII
Certain defects in the international application

a) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D5 are not mentioned in the description, nor are these documents identified therein.

Furthermore, for the purpose of examining inventive step in the regional phase, the applicant should supply the appropriate offices with a full reference for the information described on lines 19-24 of page 45.

Re Item VIII
Certain observations on the international application

a) The Applicant is reminded that the claims must be comprehensible from the technical point of view and clearly define the object of the invention, that is to say indicate all the essential features thereof (Rule 6 PCT). The subject-matter of Claims 1-31 and 37-46 does not fulfil this condition, as the claimed nucleic acid is only defined by the name of the encoded protein "GPI-PLD" or "mature human wild-type GPI-PLD", or by a functional feature without disclosing any technical feature which unambiguously characterizes the claimed subject-matter. A gene, being a chemical product, should be clearly defined by its formula i.e. its nucleotide sequence.

b) The term "incorporated by reference" on page 46 lines 2-3 should be removed. A patent application must be self understanding; the objected term renders the scope of the application obscure (Art. 5 and 6, Rule 9.1(iv) PCT).

c) The term "product of GPI-PLD action" in claims 28, 29 and 31 is unclear. The products should be defined, insofar as they are present within the description (Article 6 PCT).

d) Similarly, the "binding agent" of claims 29 and 31 lacks the technical features enabling one skilled in the art to identify such an agent. The technical features should therefore be added (e.g. specific antibody; Article 6 PCT).

Claims:

1. Glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for use in a method of medical treatment.

2. The GPI-PLD of claim 1, wherein the GPI-PLD is simultaneously or sequentially administered with apoliprotein A1.

3. A nucleic acid molecule encoding GPI-PLD for use in a method of medical treatment.

4. Use of GPI-PLD for the preparation of a medicament for the treatment of conditions that respond to GPI-PLD or which are characterised by reduced levels of GPI-PLD as compared to a normal patient.

5. Use of the presence or amount of GPI-PLD in a sample from a patient in the diagnosis of diabetes or diabetic complications, disorders involving pancreatectomies, or a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock.

6. The use of claim 5, wherein the presence or amount of GPI-PLD is determined by measuring one of its biological activities.

7. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of diabetes or diabetic complications.

8. The use of claim 7, wherein the diabetes is an insulin dependent form of diabetes.

9. The use of claim 7 or claim 8, wherein the diabetes is Type I or Type II diabetes.

10. The use of claim 7, wherein the complications of diabetes are due to insulin resistance.

11. The use of any one of claims 7 to 10, wherein the medicament further comprises insulin, a glucose sparing or insulin enhancing drug, an α-glucosidase inhibitor or drug to treat insulin sensitivity, a P and/or A-type inositolphosphoglycan (IPG) and/or an IPG antagonist.

12. Use of a nucleic acid molecule encoding GPI-PLD, in the preparation of a medicament for the treatment of diabetes or diabetic complications.

13. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of liver dysfunction or disorders involving pancreatectomies.

14. The use of claim 13, wherein the medicament further comprises apolipoprotein A1.

15. Use of a nucleic acid molecule encoding GPI-PLD in the preparation of a medicament for the treatment of liver dysfunction.

16. The use of any one of the preceding claims, wherein the liver dysfunction is characterised by reduced levels of apolipoprotein A1 and/or GPI PLD and/or apolipoprotein A1/GPI-PLD complex as compared to a normal patient.

17. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of a condition mediated by a

product of an infectious organism which is capable of inhibiting GPI-PLD.

18. The use of claim 17, wherein the condition is mediated by an endotoxin.

19. The use of claim 18, wherein the endotoxin is a glycolipid from a *Mycobacterium* or gram negative bacteria.

20. The use of any one of claims 17 to 19, wherein the condition is septic shock.

21. Use of a nucleic acid molecule encoding glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD.

22. A cell line transformed with nucleic acid encoding GPI-PLD, and capable of expressing and secreting GPI-PLD, for use in a method of medical treatment.

23. The cell line of claim 22, wherein the cell line is capable of producing apolipoprotein A1.

24. The use of the cell line of claim 22 or claim 23, in the preparation of a medicament for treatment of diabetes or diabetic complications, liver dysfunction or disorders involving pancreatectomies, or a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock.

25. A pharmaceutical composition comprising a nucleic acid molecule encoding a GPI-PLD protein.

26. A pharmaceutical composition comprising a GPI-PLD protein.

27. The composition of claim 22, further comprising apolipoprotein A1.

28. A method of diagnosing a condition selected from diabetes or diabetic complications, disorders involving pancreatectomies, a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising:

determining the amount of GPI-PLD or a product of GPI-PLD action in a sample from a patient and correlating the amount to standards to determine whether the patient has or is at risk from said condition.

29. The method of claim 28, which comprises the steps of:

(a) contacting a biological sample obtained from the patient with a solid support having immobilised thereon a binding agent having binding sites specific for GPI-PLD or the product of GPI-PLD action;

(b) contacting the solid support with one or more labelled developing agents capable of binding to unoccupied binding sites, bound GPI-PLD or product, or occupied binding sites; and,

(c) detecting the label of the developing agents specifically binding in step (b) to obtain a value representative of the amount of GPI-PLD or the product of GPI-PLD action in the sample.

30. The method of claim 28, wherein the amount of GPI-PLD in the sample is determined by measuring GPI-PLD activity.

31. A method of diagnosing a condition selected from diabetes or diabetic complications, liver dysfunction or disorders involving pancreatectomies, a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising:

(a) contacting a biological sample obtained from the patient with a solid support having immobilised thereon a binding agent having binding sites specific for GPI-PLD or the product of GPI-PLD action;

(b) contacting the solid support with one or more labelled developing agents capable of binding to unoccupied binding sites, bound GPI-PLD or product, or occupied binding sites; and,

(c) detecting the label of the developing agents specifically binding in step (b) to obtain a value representative of the amount of GPI-PLD or the product of GPI-PLD action in the sample.

32. The method of any one of claims 29 to 31, wherein the product of GPI-PLD action are acyl-IPGs or IPGs.

33. An isolated or substantially isolated GPI-PLD protein with an amino acid sequence as shown in Figure 3.

34. An isolated nucleic acid sequence encoding a GPI-PLD as shown in any one of Figures 4 to 6.

35. An isolated nucleic acid sequence encoding a GPI-PLD, with greater than 90% identity with any one of the nucleic acid sequences shown in Figures 4 to 6.

36. An expression vector comprising nucleic acid sequence encoding a GPI-PLD protein as shown in any one of Figures 4 to 6.

37. A variant GPI-PLD polypeptides differing in amino acid sequence in the region corresponding to amino acid residues 689-692 inclusive (RRFS) of human wild-type GPI-PLD.

38. The variant of claim 36 which comprises a substitution in the region corresponding to amino acids 689-692 of mature human wild-type GPI-PLD.

39. The variant of claim 38, wherein the substitution changes the serine residue at position 692 to an amino acid other than serine or threonine.

40. The variant of any one of claims 37 to 39 for use in a method of medical treatment.

41. An isolated nucleic acid molecule encoding the variant GPI-PLD polypeptide of any one of claims 37 to 39.

42. The nucleic acid of any one of claims 37 to 39 for use in a method of medical treatment.

43. An expression vector comprising the nucleic acid molecule of claim 41, operably linked to control sequences to direct its expression.

44. A host cell transformed with the nucleic acid molecule of claim 41 encoding a GPI-PLD variant polypeptide.

45. A method of producing a variant GPI-PLD polypeptides which comprises culturing the host cells of claim 44 so that the variant GPI-PLD polypeptide is expressed and isolating the polypeptide thus produced.

46. The method of claim 45 which comprises the further step of then formulating the variant GPI-PLD polypeptide in a composition.

5

PATENT COOPERATION TREATY

REC'D 23 MAR 2001
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SJK/BP5827712		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04399	23/12/1999	24/12/1998
International Patent Classification (IPC) or national classification and IPC C12N15/55		
Applicant UNIVERSITY COLLEGE LONDON et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/06/2000	21.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Page, M Telephone No. +49 89 2399 7322

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04399

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-46 as originally filed

Claims, No.:

1-46 as received on 28/02/2001 with letter of 28/02/2001

Drawings, sheets:

1/20-20/20 as originally filed

Sequence listing part of the description, pages:

1-57 (SEQ ID NOs. 1-30), filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 33-36.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 33-36.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-32, 40, 42
	No:	Claims 37-39, 41, 43-46

Inventive step (IS)	Yes:	Claims	1-32, 40, 42
	No:	Claims	37-39, 41, 43-46
Industrial applicability (IA)	Yes:	Claims	1-32, 37-46
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The application concerns the provision of polynucleotides and polypeptides corresponding to glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) for therapeutic application, methods for diagnosing conditions associated with altered GPI-PLD levels and variant polypeptide and polynucleotide sequences. Several GPI-PLD variants are known in the art, but they have not been disclosed as being suitable for therapeutic use.

Re Item II
Priority

After considering the priority documents, the document cited "P, X" in the search report is not considered relevant for the examination of novelty and inventive step.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The subject matter of **claims 33-36** was not examined due to the non-establishment of a search report for these claims. See International Search Report for details.

Re Item IV
Lack of Unity of Invention

The application concerns the provision of GPI-PLD polypeptides and polynucleotides for use in medical treatment, the provision of supposedly novel GPI-PLD variants (not explicitly for use in medical treatment) and diagnostic methods associated with the polypeptides. Multiple (variant) polynucleotide and polypeptide sequences for GPI-PLD are known in the art (D2 Figs. 9 and 11), as are diagnostic methods for their quantification (D1 page 76 GPI-PLD activity activities in different patient groups). Therefore, the three inventive concepts are not linked to form a common underlying inventive concept as it is considered that there is no special technical feature present. The application does not comply with the requirements for unity of invention (Article 34(3) and Rules 13 and 68 PCT) and the subject matter of the application is therefore

considered to relate not to one, but to 3 separate inventions as follows:

Invention I Claims 1-4, 7-27, 40 and 42: GPI-PLD polypeptides and polynucleotides for use in medical treatment.

Invention II Claims 5, 6 and 28-32: Methods of diagnosis, the methods comprising the determination of GPI-PLD activity in a sample.

Invention III Claims 37-39, 41 and 43-46: Variant GPI-PLD polypeptides and polynucleotides.

N.B. The change in dependency of claims 43-46 has led to the reassessment of the said claims, not only with regard to which invention they belong to, but also with regard to novelty and inventive step.

N.B. the use of the term "invention" here in no way implies recognition of an inventive step for the subject-matter.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

D1: MAGUIRE, G.A. & GOSSNER, A.: 'Glycosyl phosphatidyl inositol phospholipase D activity in human serum' ANNALS OF CLINICAL BIOCHEMISTRY, vol. 32, no. 1, January 1995 (1995-01), pages 74-78, XP000864653

D2: EP-A-0 477 739 (F. HOFFMANN-LA ROCHE AG) 1 April 1992 (1992-04-01) cited in the application

D3: SCALLON, B.J. ET AL.: 'Primary Structure and Functional Activity of a Phosphatidylinositol-Glycan-Specific Phospholipase D' SCIENCE, vol. 252, no. 5004, 19 April 1991 (1991-04-19), pages 446-448, XP000293055 cited in the application

D4: HOENER, M.C. & BRODBECK, U.: 'Phosphatidylinositol-glycan-specific

phospholipase D is an amphiphilic glycoprotein that in serum is associated with high-density lipoproteins' EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 206, no. 3, June 1992 (1992-06), pages 747-757, XP000913778 cited in the application

D5: HUANG, L.C ET AL.: 'Chiroinositol Deficiency and Insulin Resistence. III. Acute Glycogenic and Hypoglycemic Effects of Two Inositol Phosphoglycan Insulin Mediators in Normal and Streptozotocin-Diabetic Rats in Vivo' ENDOCRINOLOGY, vol. 132, no. 2, January 1993 (1993-01), pages 652-657, XP002050432

2) **Novelty - Art.33(1) and (2) PCT**:

Invention I

Claims 1-4, 7-27, 40 and 42 appear to be novel in light of the cited prior art. GPI-PLD for use in medical treatment has not been previously disclosed.

Invention II

Claims 5, 6 and 28-30 appear to be novel in light of the cited prior art. Although D1 discloses the use of an assay for determining the activity of GPI-PLD in human serum and correlates this activity to pathologies of the liver characterised by reduced GPI-PLD levels (D1 page 76 GPI- PLD activity activities in different patient groups), no mention is made of diabetic disorders, pancreatectomies or conditions mediated by the product of infectious organisms that inhibit GPI-PLD.

Claims 31 and 32 also appears to be novel in light of the cited prior art. The technical features of the assay provided in claim 31 are not disclosed in D1.

Invention III

Claims 37-39, 41 and 43-46 cannot be acknowledged as being novel as no sequence is defined in which the provided changes are to be found. The claims could thus apply to any GPI-PLD polypeptide sequence and thus lack novelty in light of e.g. D1 and D2, which disclose further GPI-PLD sequences.

3) **Inventive Step - Art.33(1) and (3) PCT**:

The following comments on inventive step are confined to subject matter which could be acknowledged as being novel, or for which novelty could potentially be restored as outlined supra.

Invention I

The closest prior art is D2, which provides the polynucleotide and polypeptide sequences of bovine GPI-PLD and human liver and pancreas GPI-PLD (D2 Figs. 5, 9 and 11).

In light of the prior art, the technical problem can be regarded as the provision of GPI-PLD for medical use.

The technical problem is solved by the subject matter of claims 1-4, 7-27, 40 and 42, which provide GPI-PLD for therapeutic use.

In light of the cited prior art, there does not appear to be any motivation to prepare GPI-PLD for therapeutic purposes. Although it is known that one of the products of this enzyme, namely inositol phosphoglycan, mediates insulin action (D5 page 656 left-hand column paragraph 2), there does not appear to be any motivation to increase the level of this molecule through treatment with GPI-PLD.

Claims 1-4, 7-27, 40 and 42 therefore appear to demonstrate inventive step in light of the cited prior art.

Invention II

The closest prior art is D1, which provides a diagnostic assay for the detection of GPI-PLD in biological samples (D1 page 76 GPI-PLD activity activities in different patient groups).

In light of the prior art, the technical problem can be regarded as the provision of methods for diagnosing specific conditions in which GPI-PLD is inhibited or depleted by determining the biological activity of GPI-PLD.

The technical problem is solved by the subject matter of claims 5, 6 and 28-32, which provide an association (immuno-type) assay for GPI-PLD or for a product of GPI-PLD action, such as IPG or acyl-IPG.

In light of the cited prior art, **claims 5, 6 and 28-30** appear to be inventive. The prior art does not disclose the diagnosis of the listed conditions using GPI-PLD concentrations or activities.

Claims 31 and 32 also appears to be inventive in light of the cited prior art: Other assays provided by the art rely on the cleavage of enzymes from insoluble supports by GPI- PLD and the subsequent measurement of enzyme activity (e.g. D1 page 75 Assay for GPI-PLD activity in human serum). The assay provided in claim 31, however, relies on the immobilisation of the enzyme on a solid support using a GPI-PLD binding protein and quantifying unoccupied binding sites.

Invention III

The closest prior art is D2, which provides 3 GPI-PLD polypeptide and polynucleotide sequence pairs (D2 Figs. 5, 9 and 11).

In light of the prior art, the technical problem can be regarded as the provision of further variant GPI-PLD polypeptide and polynucleotide sequences.

The technical problem is solved by the subject matter of claims 37-39, 41 and 43-46.

Even if novelty had been restored to claims 36-38 and 40 by defining the sequence for which protection is sought, it cannot be seen how the subject matter of **claims 37-39, 41 and 43-46** could be regarded as inventive. In the absence of any form of functional statement or support in the form of specific examples of these variants, it is not possible to acknowledge inventive step.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 47565	23/09/1999	18/03/1999	18/03/1998 21/05/1998

Document relevant to claims 28, 29 and 31.

Re Item VII
Certain defects in the international application

a) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D5 are not mentioned in the description, nor are these documents identified therein.

Furthermore, for the purpose of examining inventive step in the regional phase, the applicant should supply the appropriate offices with a full reference for the information described on lines 19-24 of page 45.

Re Item VIII
Certain observations on the international application

a) The Applicant is reminded that the claims must be comprehensible from the technical point of view and clearly define the object of the invention, that is to say indicate all the essential features thereof (Rule 6 PCT). The subject-matter of Claims 1-31 and 37-46 does not fulfil this condition, as the claimed nucleic acid is only defined by the name of the encoded protein "GPI-PLD" or "mature human wild-type GPI-PLD", or by a functional feature without disclosing any technical feature which unambiguously characterizes the claimed subject-matter. A gene, being a chemical product, should be clearly defined by its formula i.e. its nucleotide sequence.

b) The term "incorporated by reference" on page 46 lines 2-3 should be removed. A patent application must be self understanding; the objected term renders the scope of the application obscure (Art. 5 and 6, Rule 9.1(iv) PCT).

c) The term "product of GPI-PLD action" in claims 28, 29 and 31 is unclear. The products should be defined, insofar as they are present within the description (Article 6 PCT).

d) Similarly, the "binding agent" of claims 29 and 31 lacks the technical features enabling one skilled in the art to identify such an agent. The technical features should therefore be added (e.g. specific antibody; Article 6 PCT).

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

Receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* SJK/BP5827712

Box No. I **TITLE OF INVENTION** GLYCOSYLPHOSPHATIDYLINOSITOL SPECIFIC PHOSPHOLIPASE D PROTEINS AND USES THEREOF

Box No. II **APPLICANT**

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

UNIVERSITY COLLEGE LONDON
GOWER STREET
LONDON WC1E 6BT
GB

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country) of nationality:* GB

State *(that is, country) of residence:* GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III **FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

JULIAN SCHOFIELD
59 MOORGREEN HOUSE
WYNYATT STREET
LONDON EC1V 7JA
GB

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(if this check-box is marked, do not fill in below.)*

State *(that is, country) of nationality:* GB

State *(that is, country) of residence:* GB

This person is applicant for the purposes of: ☐ all designated ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV **AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

KIDDLE, SIMON J. and others
MEWBURN ELLIS
YORK HOUSE
23 KINGSWAY
LONDON WC2B 6HP
GB

Telephone No. 0117 9266411

Facsimile No. +44 20 7240 9339

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

If none of the fo[…]ing sub-boxes is used, this sheet is not to be [in]cluded in the request.

Name and address: *(Family name follow[ed by] given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is t[he a]pplicant's State (that is, country) of residence if no State of residence is indicated below.)*

RADEMACHER THOMAS WILLIAM
Foxcombe
The Ridgeway
Boars Hill
Oxford OX1 5EY
GB

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(if this check-box is marked, do not fill in below.)*

State *(that is, country) of nationality:* US

State *(that is, country) of residence:* GB

This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.))*

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only *(if this check-box is marked, do not fill in below.)*

State *(that is, country) of nationality:*

State *(that is, country) of residence:*

This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.))*

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only *(if this check-box is marked, do not fill in below.)*

State *(that is, country) of nationality:*

State *(that is, country) of residence:*

This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.))*

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only *(if this check-box is marked, do not fill in below.)*

State *(that is, country) of nationality:*

State *(that is, country) of residence:*

This person is applicant for the purposes of: ☐ all designated states ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

[X] **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

[X] **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

[X] **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

[X] **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)*

.....

National Patent *(if other kind of protection desired, specify on dotted line)*:

[X] **AE** United Arab Emirates
[X] **AL** Albania.....
[X] **AM** Armenia.....
[X] **AT** Austria.....
[X] **AU** Australia.....
[X] **AZ** Azerbaijan
[X] **BA** Bosnia & Herzegovina.....
[X] **BB** Barbados
[X] **BG** Bulgaria.....
[X] **BR** Brazil.....
[X] **BY** Belarus.....
[X] **CA** Canada
[X] **CH and LI** Switzerland and Liechtenstein.....
[X] **CN** China.....
[X] **CU** Cuba.....
[X] **CZ** Czech Republic.....
[X] **DE** Germany.....
[X] **DK** Denmark.....
[X] **EE** Estonia.....
[X] **ES** Spain.....
[X] **FI** Finland.....
[X] **GB** United Kingdom.
[X] **GD** Grenada
[X] **GE** Georgia.....
[X] **GH** Ghana.....
[X] **GM** Gambia
[X] **HR** Croatia.....
[X] **HU** Hungary .....
[X] **ID** Indonesia
[X] **IL** Israel.....
[X] **IN** India
[X] **IS** Iceland
[X] **JP** Japan.....
[X] **KE** Kenya.....
[X] **KG** Kyrgyzstan.....
[X] **KP** Democratic People's Republic of Korea.....
[X] **KR** Republic of Korea.....
[X] **KZ** Kazakstan .....
[X] **LC** St Lucia
[X] **LK** Sri Lanka
[X] **LR** Liberia.
[X] **LS** Lesotho.....
[X] **LT** Lithuania
[X] **LU** Luxembourg
[X] **LV** Latvia
[X] **MD** Republic of Moldova .....
[X] **MG** Madagascar .....
[X] **MK** The former Yugoslav Republic of Macedonia...
[X] **MN** Mongolia .....
[X] **MW** Malawi.....
[X] **MX** Mexico.....
[X] **NO** Norway
[X] **NZ** New Zealand .....
[X] **PL** Poland .....
[X] **PT** Portugal .....
[X] **RO** Romania
[X] **RU** Russian Federation .....
[X] **SD** Sudan
[X] **SE** Sweden
[X] **SG** Singapore
[X] **SI** Slovenia .....
[X] **SK** Slovakia .....
[X] **SL** Sierra Leone
[X] **TJ** Tajikistan .....
[X] **TM** Turkmenistan .....
[X] **TR** Turkey .....
[X] **TT** Trinidad and Tobago .....
[X] **UA** Ukraine .....
[X] **UG** Uganda .....
[X] **US** United States of America.....
[X] **UZ** Uzbekistan .....
[X] **VN** Viet Nam .....
[X] **YU** Yugoslavia .....
[X] **ZA** South Africa
[X] **ZW** Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

[X] **CR** Costa Rica
[X] **DM** Dominica
[X] **TZ** Tanzania
[X] **MA** Morocco
[X] Any other state which is party to the PCT .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement.
The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

Use this box in the following cases:

I. If any of the Boxes, the space is insufficient to furnish all the information: — *In such case, write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;*

in particular:

(i) *if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:* — *in such case, write "Continuation of Box III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this box is the applicant's state (that is, country) of residence if no state of residence is indicated below;*

(ii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:* — *in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

(iii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:* — *in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

(iv) *if, in addition to the agent(s) indicated in Box No. IV, there are further agents:* — *in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

(v) *if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":* — *in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

(vi) *if, in Box No. VI, there are more than three earlier applications whose priority is claimed:* — *in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*

(vii) *if, in Box No. VI, the earlier application is an ARIPO application:* — *in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: — *in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each state so excluded.*

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: — *in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.*

Continuation of Box IV

ARMITAGE, IAN M.
BRASNETT, ADRIAN H.
CALDERBANK, T. ROGER
CARTER, STEPHEN
COLEIRO, RAYMOND
CRIPPS, JOANNA E
FORD, MICHAEL F.
GURA, H. ALAN
HACKNEY, NIGEL J.
HARRISON, DAVID C.
KIDDLE, SIMON J.
KREMER, SIMON M.
LINN, S. JONATHAN
LYONS, JUNE, M.
NICHOLLS, KATHRYN M.
O'BRIEN, CAROLINE J.
PAGET, HUGH C.E.
PAGET, HUGH C.E.
SANDERSON, MICHAEL J.
STONER, G. PATRICK
STUART, IAN
.WALTON, SEÁN M
WATSON, ROBERT J.

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 24 December 1998	9828712.1	GB		
item (2) 24 December 1998	9828715.4	GB		
item (3) 24 December 1998	9828713.9	GB		

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s):* (3)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the supplemental box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA /

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets	
request	:5
description (excluding sequence listing part)	:46
claims	:6
abstract	:1
drawings	:18
sequence listing part of description	:0
Total number of sheets	**:76**

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☒ priority document(s) *identified in Box No. VI as item*(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganisms or other biological matter
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: 23/77

Figure of the drawings which should accompany the abstract: 0

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

.....

KIDDLE, SIMON J.
APPOINTED AGENT

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

DESIGNATION OF STATES

Regional Patent

[X] **AP** **ARIPO Patent:** GH Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** Tanzania, **UG** Uganda, **ZW** Zimbabwe and any other State which is a Contracting State of the Harare Protocol and of the PCT

[X] **EP** **European Patent: AT** Austria, **BE** Belgium, **CH** and **LI** Switzerland and Liechtenstein, **CY** Cyprus, DE Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden and any other State which is a Contracting State of the European Patent Convention and of the PCT

[X] **OA** **OAPI Patent: BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea,**GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT

[X] **EA** **Eurasian Patent: AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan and any other state which is a member state of EAPC and a Contracting State of the PCT

National Patent

- [X] **AE** United Arab Emirates
- [X] **AL** Albania
- [X] **AM** Armenia
- [X] **AT** Austria
- [X] **AU** Australia
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- [X] **KR** Republic of Korea
- [X] **KZ** Kazakstan
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- [X] **TR** Turkey
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- [X] **TZ** Tanzania
- [X] **UA** Ukraine
- [X] **UG** Uganda
- [X] **US** United States of America
- [X] **UZ** Uzbekistan
- [X] **VN** Vietnam
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- [X] **ZA** South Africa
- [X] **ZW** Zimbabwe

Check-boxes reserved for States which have become party to the PCT after issuance of this sheet

[ ] ..... [ ] .....

Note: this sheet lists all states known to be PCT Contracting States at 8 October 1999

Mewburn Ellis, October 1999
3designx.frm

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For rec[redacted] Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: SJK/BP5827712

Applicant: UNIVERSITY COLLEGE LONDON

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE..... £55 T

2. SEARCH FEE ..... £638 S

International search to be carried out by _____
(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 76 sheets.

first 30 sheets ..... £285 b_1

46 x £6 = £276 b_2
remaining sheets additional amount

Add amounts entered at b_1 and b_2 and enter total at B.... £561 B

Designation Fees

The international application contains 81 designations.

10 x £65 = £650 D
number of designation fees payable *(maximum 10)* amount of designation fee

Add amounts entered at B and D and enter total at I ..... £1211 I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT (if applicable) ..... £66 P

5. TOTAL FEES PAYABLE
Add amounts entered at T, S, I and P, and enter total in the TOTAL box: £1970 TOTAL

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

- ☐ authorization to charge deposit account (see below)
- ☐ bank draft
- ☐ coupons
- ☒ cheque
- ☐ cash
- ☐ other *(specify)*
- ☐ postal money order
- ☐ revenue stamps

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ ____ ☐ is hereby authorized to charge the total fee indicated above to my deposit account.

☐ is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☐ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account Number _____ Day *(day/month/year)* _____ Signature

From the RECEIVING OFFICE

PCT

To:
Mewburn Ellis
York House
23 Kingsway
London

WC2B 6HP

RECEIVED
13 JAN 2000

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing *(day/month/year)* 11 JAN 2000

Applicant's or agents's file reference
SJK/BP5827712

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04399	23/12/1999	24/12/1998

Applicant
University College London et al

Title of the invention
Glycosylphosphatidylinositol Specific Phospholipase D Proteins And Uses Thereof

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

☒ was transmitted to the International Bureau on 11 JAN 2000

☐ has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:

☐ because the necessary national security clearance has not yet been obtained.

☐ because *(reason to be specified)*:

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

Name and mailing address of the receiving Office	Authorized officer
The Patent Office Cardiff Road, Newport South Wales NP9 1RH	Karen Mitchell
Facsimile No.	Telephone No. 01633 814384

Form PCT/RO/105 (July 1992)

Claims:

1. Glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for use in a method of medical treatment.

2. The GPI-PLD of claim 1, wherein the GPI-PLD is simultaneously or sequentially administered with apoliprotein A1.

3. A nucleic acid molecule encoding GPI-PLD for use in a method of medical treatment.

4. Use of GPI-PLD for the preparation of a medicament for the treatment of conditions that respond to GPI-PLD or which are characterised by reduced levels of GPI-PLD as compared to a normal patient.

5. Use of the presence or amount of GPI-PLD in a sample from a patient in the diagnosis of diabetes or diabetic complications, disorders involving pancreatectomies, or a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock.

6. The use of claim 5, wherein the presence or amount of GPI-PLD is determined by measuring one of its biological activities.

7. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of diabetes or diabetic complications.

8. The use of claim 7, wherein the diabetes is an insulin dependent form of diabetes.

9. The use of claim 7 or claim 8, wherein the diabetes is Type I or Type II diabetes.

10. The use of claim 7, wherein the complications of diabetes are due to insulin resistance.

11. The use of any one of claims 7 to 10, wherein the medicament further comprises insulin, a glucose sparing or insulin enhancing drug, an α-glucosidase inhibitor or drug to treat insulin sensitivity, a P and/or A-type inositolphosphoglycan (IPG) and/or an IPG antagonist.

12. Use of a nucleic acid molecule encoding GPI-PLD, in the preparation of a medicament for the treatment of diabetes or diabetic complications.

13. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of liver dysfunction or disorders involving pancreatectomies.

14. The use of claim 13, wherein the medicament further comprises apolipoprotein A1.

15. Use of a nucleic acid molecule encoding GPI-PLD in the preparation of a medicament for the treatment of liver dysfunction.

16. The use of any one of the preceding claims, wherein the liver dysfunction is characterised by reduced levels of apolipoprotein A1 and/or GPI PLD and/or apolipoprotein A1/GPI-PLD complex as compared to a normal patient.

17. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of a condition mediated by a

product of an infectious organism which is capable of inhibiting GPI-PLD.

18. The use of claim 17, wherein the condition is mediated by an endotoxin.

19. The use of claim 18, wherein the endotoxin is a glycolipid from a *Mycobacterium* or gram negative bacteria.

20. The use of any one of claims 17 to 19, wherein the condition is septic shock.

21. Use of a nucleic acid molecule encoding glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD.

22. A cell line transformed with nucleic acid encoding GPI-PLD, and capable of expressing and secreting GPI-PLD, for use in a method of medical treatment.

23. The cell line of claim 22, wherein the cell line is capable of producing apolipoprotein A1.

24. The use of the cell line of claim 22 or claim 23, in the preparation of a medicament for treatment of diabetes or diabetic complications, liver dysfunction or disorders involving pancreatectomies, or a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock.

25. A pharmaceutical composition comprising a nucleic acid molecule encoding a GPI-PLD protein.

26. A pharmaceutical composition comprising a GPI-PLD protein.

27. The composition of claim 22, further comprising apolipoprotein A1.

28. A method of diagnosing a condition selected from diabetes or diabetic complications, disorders involving pancreatectomies, a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising:

determining the amount of GPI-PLD or a product of GPI-PLD action in a sample from a patient and correlating the amount to standards to determine whether the patient has or is at risk from said condition.

29. The method of claim 28, which comprises the steps of:

(a) contacting a biological sample obtained from the patient with a solid support having immobilised thereon a binding agent having binding sites specific for GPI-PLD or the product of GPI-PLD action;

(b) contacting the solid support with one or more labelled developing agents capable of binding to unoccupied binding sites, bound GPI-PLD or product, or occupied binding sites; and,

(c) detecting the label of the developing agents specifically binding in step (b) to obtain a value representative of the amount of GPI-PLD or the product of GPI-PLD action in the sample.

30. The method of claim 28, wherein the amount of GPI-PLD in the sample is determined by measuring GPI-PLD activity.

31. A method of diagnosing a condition selected from diabetes or diabetic complications, liver dysfunction or disorders involving pancreatectomies, a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising:

(a) contacting a biological sample obtained from the patient with a solid support having immobilised thereon a binding agent having binding sites specific for GPI-PLD or the product of GPI-PLD action;

(b) contacting the solid support with one or more labelled developing agents capable of binding to unoccupied binding sites, bound GPI-PLD or product, or occupied binding sites; and,

(c) detecting the label of the developing agents specifically binding in step (b) to obtain a value representative of the amount of GPI-PLD or the product of GPI-PLD action in the sample.

32. The method of any one of claims 29 to 31, wherein the product of GPI-PLD action are acyl-IPGs or IPGs.

33. An isolated or substantially isolated GPI-PLD protein with an amino acid sequence as shown in Figure 3.

34. An isolated nucleic acid sequence encoding a GPI-PLD as shown in any one of Figures 4 to 6.

35. An isolated nucleic acid sequence encoding a GPI-PLD, with greater than 90% identity with any one of the nucleic acid sequences shown in Figures 4 to 6.

36. An expression vector comprising nucleic acid sequence encoding a GPI-PLD protein as shown in any one of Figures 4 to 6.

37. A variant GPI-PLD polypeptides differing in amino acid sequence in the region corresponding to amino acid residues 689-692 inclusive (RRFS) of human wild-type GPI-PLD.

38. The variant of claim 36 which comprises a substitution in the region corresponding to amino acids 689-692 of mature human wild-type GPI-PLD.

39. The variant of claim 38, wherein the substitution changes the serine residue at position 692 to an amino acid other than serine or threonine.

40. The variant of any one of claims 37 to 39 for use in a method of medical treatment.

41. An isolated nucleic acid molecule encoding the variant GPI-PLD polypeptide of any one of claims 37 to 39.

42. The nucleic acid of any one of claims 37 to 39 for use in a method of medical treatment.

43. An expression vector comprising the nucleic acid molecule of claim 41, operably linked to control sequences to direct its expression.

44. A host cell transformed with the nucleic acid molecule of claim 41 encoding a GPI-PLD variant polypeptide.

45. A method of producing a variant GPI-PLD polypeptides which comprises culturing the host cells of claim 44 so that the variant GPI-PLD polypeptide is expressed and isolating the polypeptide thus produced.

46. The method of claim 45 which comprises the further step of then formulating the variant GPI-PLD polypeptide in a composition.

5

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

KIDDLE, Simon, J.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

RECEIVED
21 FEB 2000

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
10 February 2000 (10.02.00)	
Applicant's or agent's file reference	**International application No.**
SJK/BP5827712	PCT/GB99/04399

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

UNIVERSITY COLLEGE LONDON (for all designated States except US)
SCHOFIELD, Julian et al (for US)

International filing date	:	23 December 1999 (23.12.99)
Priority date(s) claimed	:	24 December 1998 (24.12.98) 24 December 1998 (24.12.98) 24 December 1998 (24.12.98)
Date of receipt of the record copy by the International Bureau	:	26 January 2000 (26.01.00)
List of designated Offices	:	

AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
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The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

F. Gateau

Telephone No. (41-22) 338.83.38

NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
10 February 2000 (10.02.00)	
Applicant's or agent's file reference	**International application No.**
SJK/BP5827712	PCT/GB99/04399

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [ ] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

KIDDLE, Simon, J.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

RECEIVED
- 6 MAR 2000

Date of mailing (day/month/year) 28 February 2000 (28.02.00)	
Applicant's or agent's file reference SJK/BP5827712	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/04399	International filing date (day/month/year) 23 December 1999 (23.12.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 24 December 1998 (24.12.98)
Applicant UNIVERSITY COLLEGE LONDON et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
24 Dece 1998 (24.12.98)	9828712.1	GB	26 Janu 2000 (26.01.00)
24 Dece 1998 (24.12.98)	9828715.4	GB	26 Janu 2000 (26.01.00)
24 Dece 1998 (24.12.98)	9828713.9	GB	26 Janu 2000 (26.01.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
Tessadel PAMPLIEGA

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
KIDDLE, Simon, J.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

RECEIVED
14 JUL 2000

Date of mailing (day/month/year) 06 July 2000 (06.07.00)	
Applicant's or agent's file reference SJK/BP5827712	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/GB99/04399	23 December 1999 (23.12.99)	24 December 1998 (24.12.98)

Applicant
UNIVERSITY COLLEGE LONDON et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,
   GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,
   OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   06 July 2000 (06.07.00) under No. WO 00/39285

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

The demand must be filed directly with the competent International Preliminary Examining Authority. The one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/_____

PCT

DEMAND

COPY

CHAPTER II

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elect all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: SJK/BP5827712

International application No.	International filing date *(day/month/year)*	(Earliest Priority date *(day/month/year)*
PCT/GB99/04399	23 December 1999 (23.12.99)	24 December 1998 (24.12.98)

Title of invention: GLYCOSYLPHOSPHATIDYLINOSITOL SPECIFIC PHOSPHOLIPASE D PROTEINS AND USES THEREOF

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

UNIVERSITY COLLEGE LONDON
GOWER STREET
LONDON WC1E 6BT
GB

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(i.e. country)* of nationality: GB

State *(i.e. country)* of residence: GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SCHOFIELD JULIAN
59 MOORGREEN HOUSE
WYNYATT STREET
LONDON EC1V 7JA
GB

State *(i.e. country)* of nationality: GB

State *(i.e. country)* of residence: GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

RADEMACHER THOMAS WILLIAM
Foxcombe
The Ridgeway
Boars Hill
Oxford OX1 5EY
GB

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: GB

☐ Further applicants are indicated on a continuation sheet.

International application No.
GB99/04399

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

KIDDLE, SIMON J.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
GB

Telephone No.: 020 7240 4405

Facsimile No.: 020 7240 9339

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1 The applicant wishes the international preliminary examination **to start on the basis of:**

[X] the international application as originally filed

the description [ ] as originally filed.
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examination Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed, or where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

[X] which is the language in which the international application was filed.

[ ] which is the language of a translation furnished for the purposes of international search.

[ ] which is the language of publication of the international application.

[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

International application No.
PCT/GB99/04399

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination

				For International Preliminary Examining Authority use only: received	not received
1.	translation of international application	: 0	sheets	☐	☐
2.	amendments under Article 34	: 0	sheets	☐	☐
3.	copy (or, where required, translation) of amendments under Article 19	: 0	sheets	☐	☐
4.	copy (or, when required, translation) of	0	sheets	☐	☐
5.	letter	: 0	sheets	☐	☐
6.	other *(specify)*	: 0	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Simon Fiddy

APPOINTED AGENT

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
SJK/BP5827712		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/04399	23/12/1999	24/12/1998
Applicant		
UNIVERSITY COLLEGE LONDON et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readble form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

HUMAN GLYCOSYLPHOSPHATIDYLINOSITOL SPECIFIC PHOSPHOLIPASE D VARIANTS AND USES THEREOF

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

☐ None of the figures.

☐ as suggested by the applicant.

☒ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

[redacted]ional application No.
PCT/GB 99/04399

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-31, and 36-45 completely

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-31 and 36-45 completely

A glycosylphosphatidylinositol specific phospholipase D (GPI-PLD) and a nucleic acid encoding GPI-PLD for the use in a method of medical treatment; the use of GPI-PLD or a nucleic acid encoding GPI-PLD for the preparation of a medicament for the treatment of diabetes/diabetic complications, liver dysfunction/disorders involving pancreatectomies and a condition mediated by a product of an infectious organism being capable of inhibiting GPI-PLD; the use of the presence or amount of GPI-PLD in a sample derived from a patient in diagnosis; a diagnostic method for diabetes/diabetic complications, liver dysfunction/disorders involving pancreatectomies and a condition mediated by a product of an infectious organism being capable of inhibiting GPI-PLD comprising the determination of the amount of GPI-PLD or a product of GPI-PLD action in a sample derived from a patient; a cell line transformed with a nucleic acid encoding GPI-PLD; the use of said cell line for the preparation of said medicament; a pharmaceutical composition comprising GPI-PLD or a nucleic acid encoding GPI-PLD; a GPI-PLD variant differing in amino acid sequence at positions 689-692 of human wild-type GPI-PLD and a nucleic acid encoding said GPI-PLD variant for the use in a method of medical treatment; an expression vector comprising said nucleic acid encoding said GPI-PLD variant; a host cell transformed with said nucleic acid encoding said GPI-PLD variant; a method of producing said GPI-PLD variant.

2. Claims: 32-35 partially

An isolated human GPI-PLD protein corresponding to clone a1 having an amino acid sequence as shown in Figure 3 and an isolated nucleic acid sequence encoding said GPI-PLD protein as shown in Figure 4; an expression vector comprising said nucleic acid sequence; an isolated nucleic acid sequence encoding a GPI-PLD protein having greater than 90% identity with the nucleic acid sequence as shown in Figure 4.

3. Claims: 32-35 partially

An isolated human GPI-PLD protein corresponding to clone b2 having an amino acid sequence as shown in Figure 3 and an isolated nucleic acid sequence encoding said GPI-PLD protein as shown in Figure 5; an expression vector comprising said nucleic acid sequence; an isolated nucleic acid sequence encoding a GPI-PLD protein having greater than 90% identity with the nucleic acid sequence as shown in Figure 5.

4. Claims: 32-35 partially

An isolated human GPI-PLD protein corresponding to clone d3

having an amino acid sequence as shown in Figure 3 and an isolated nucleic acid sequence encoding said GPI-PLD protein as shown in Figure 6; an expression vector comprising said nucleic acid sequence; an isolated nucleic acid sequence encoding a GPI-PLD protein having greater than 90% identity with the nucleic acid sequence as shown in Figure 6.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/55, 9/16, C12Q 1/34, G01N 33/48, A61K 38/46, 48/00, A61P 1/18, 1/16, 31/00

A3

(11) International Publication Number: WO 00/39285

(43) International Publication Date: 6 July 2000 (06.07.00)

(21) International Application Number: PCT/GB99/04399

(22) International Filing Date: 23 December 1999 (23.12.99)

(30) Priority Data:

9828712.1	24 December 1998 (24.12.98)	GB
9828715.4	24 December 1998 (24.12.98)	GB
9828713.9	24 December 1998 (24.12.98)	GB

(71) Applicant *(for all designated States except US)*: UNIVERSITY COLLEGE LONDON [GB/GB]; Gower Street, London WC1E 6BT (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SCHOFIELD, Julian [GB/GB]; 59 Moorgreen House, Wynyatt Street, London EC1V 7JA (GB). RADEMACHER, Thomas, William [US/GB]; Foxcombe, The Ridgeway, Boars Hill, Oxford OX1 5EY (GB).

(74) Agents: KIDDLE, Simon, J. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
16 November 2000 (16.11.00)

(54) Title: HUMAN GLYCOSYLPHOSPHATIDYLINOSITOL SPECIFIC PHOSPHOLIPASE D VARIANTS AND USES THEREOF

(57) Abstract

Glycosyl phosphatidy linositol specific phospholipase D (GPI-PLD) proteins and their medical uses are disclosed, in particular in the treatment and diagnosis of diabetes and complications of diabetes such as insulin resistance, liver dysfunction, disorders involving pancreatectomies and conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock. The present invention further relates to variant GPI-PLD polypeptides modified at the phosphorylation site at amino acids 689-692 of the mature human wild-type protein.

```
Top: protein produced from cDNA clone A1
Mid: protein produced from Roche patent bovine liver sequence
Bot: protein produced from Roche patent human liver sequence

MSAFRLWPGLLIMLG-SLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDA
MSAFRFWSGLLMLLG-FLCPRSSPCGISTHIEIGHRALEFLHLQDGSINYKELLLRHQDA
MSAFRLWPGLLMIVMASLCHRGSSCGLSTHIEIGHRALEFLHLHNGHVNYKELLLEHQDA

YQAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFL
YQAGSVFPDSFYPSICERGQFHDVSESTHWTPFLNASVHYIRKNYPLPWDEDTEKLVAFL
YQAGTVFPDCFYPSLCKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFL

FGITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEPNFNYLA
FGITSHMVADVNWHSLGIENGFLRTMAAIDFHNSYPEAHPAGDFGGDVLSQFEFKPNYLS
PGITSHMVADVSWHSLGIEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEPNFNYLA

RRWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFL
RHWYVPAEDLLGIYRELYGRIVITKKAIVDCSYLQFLEMYAEMLAISKLYPTYSVKSPFL
RRWYVPVKDLLGIYEKLYGREVITENVIVDCSHIQFLEMYGEMLAVSKLYPSYSTKSPFL

VEQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPENPLFIACGGQQNHTQG
VEQFQEYFLGGLEDMAFWSTNIYHLTSTMLKNGTSNCNLPENP---LFITCGGQQNNTHG
VEQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCSLPENPENPLFIACGGQQNHTQG

SKMQKNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIG
SKVQKNGFHKNVTAALTKNIGKHINYTKRGVFFSVDSWTMDFLSFMYKSLERSIREMFIG
SKMQKNDFHRNLTSSLTENIDRNINYTERGVFFSVNSWTPDSMSFIYKALERNVRTMFIG

GSQLSQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRV
SSQP-LTHVSSPAASYYLSFPYTRLGWAMTSADLNQDGYGDLVVGAPGYSHPGRIHVGRV
GSQLSQKHISSPLASYFLSFPYARLGWAMTSADLNQDGYGDLVVGAPGYSRPGRIHIGRV

YLIYGNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGS
YLIYGNDLG-PRIDLDLDKEAHGILEGFQPSGRPGSAVAVLDFNVDGVPDLAVGAPSVGS
YLIYGNELGLPPVDLDLDKEAHGILEGFQPSGRFGSALAMLDFNMDGVPDLAVGAPSVGS

EQLTYKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPD-LVIGSP
EKLTYTGAVYVYFGSKQGQLSSSPNVTISCQDTYCNLGWTLLAADVDGDSEPDLFVIGSP
EQLTYKGAVYVYFGSKQGRMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPD-LVIGSP

FAPGGGKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLL
FAPGGGKQKGIVAAFYSGSSYSSREKLNVEAANWMVKGEEDFAWLGYSLHGVNVNNRTLL
FAPGGGKQKGIVAAFYSGPSLSNKEKLNVEAANWTVRGEEDFAWFGYSLHGVTVDNRTLL

LVGSPTWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGH
LAGSPTWKDTSSQGHLFRTRDEKQSPGRVYGYFPPICQSWFTISGDKAMGKLGTSLSSGH
LVGSPTWKNASRLGRLLHIRDEKKSLGRVYGYFPPNSQSWFTIVGDKAMGKLGTSLSSGH
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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/GB 99/04399

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/55 C12N9/16 C12Q1/34 G01N33/48 A61K38/46
A61K48/00 A61P1/18 A61P1/16 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q G01N A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAGUIRE, G.A. & GOSSNER, A.: "Glycosyl phosphatidyl inositol phospholipase D activity in human serum" ANNALS OF CLINICAL BIOCHEMISTRY, vol. 32, no. 1, January 1995 (1995-01), pages 74-78, XP000864653 abstract page 75, column 1, line 1 - line 39 page 76; figures 3A,C page 77, column 1, line 2 -page 78, column 1, line 13 --- -/--	4,13-16, 28,30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2000	14. 9. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VICENT, D. ET AL.: "Alterations in Skeletal Muscle Gene Expression" DIABETES, vol. 47, no. 9, September 1998 (1998-09), pages 1451-1458, XP000864657 abstract page 1454, column 1, line 9 - line 37 page 1457, column 1, line 30 - line 54 ---	4,7-12
A	EP 0 477 739 A (F. HOFFMANN-LA ROCHE AG) 1 April 1992 (1992-04-01) cited in the application abstract page 4, line 14 -page 5, line 8 page 7, line 52 -page 11, line 30; tables 1,2 page 20 -page 21; claims 1,2,5-17	1-31
Y	page 31 -page 37; figures 9,10 ---	36-45
A	SCALLON, B.J. ET AL.: "Primary Structure and Fucntional Acitvity of a Phosphatidylinositol-Glycan-Specific Phospholipase D" SCIENCE, vol. 252, no. 5004, 19 April 1991 (1991-04-19), pages 446-448, XP000293055 cited in the application abstract page 446, column 2, line 3 -page 447, column 1, line 2	1-31
Y	page 447, column 1, line 26 - line 30 page 447, column 1, line 36 -column 2, line 6; figure 3 ---	36-45
P,X	WO 99 47565 A (RADEMACHER GROUP LIMITED) 23 September 1999 (1999-09-23) abstract page 23, line 16 -page 24, line 7 page 24, line 18 - line 29 page 31 -page 35; examples 3,4 page 43-46; claims 1-5,10-14,16-19,22 page 48 -page 50; figures 2-4 --- -/--	28,29,31

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TSANG, T.C. ET AL.: "Isolation and expression of two human glycosylphosphatidylinositol phospholipase D (GPI-PLD) cDNAs" FASEB JOURNAL, vol. 6, April 1992 (1992-04), page A1922 XP000907489 cited in the application abstract no.: 5707 the whole document	1-31, 36-45
A	-& EMBL Database, Heidelberg, FRG accession number L11702 07 September 1993 TSANG, T.C. ET AL: "Human phospholipase D mRNA, complete cds" XP002141248 cited in the application the whole document	1-31, 36-45
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(57) Abstract

Glycosyl phosphatidy linositol specific phospholipase D (GPI–PLD) proteins and their medical uses are disclosed, in particular in the treatment and diagnosis of diabetes and complications of diabetes such as insulin resistance, liver dysfunction, disorders involving pancreatectomies and conditions mediated by a product of an infectious organism which is capable of inhibiting GPI–PLD, such as septic shock. The present invention further relates to variant GPI–PLD polypeptides modified at the phosphorylation site at amino acids 689–692 of the mature human wild–type protein.

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Glycosylphosphatidylinositol Specific Phospholipase D Proteins and Uses Thereof

Field of the Invention

The present invention relates to glycosylphosphatidylinositol specific phospholipase D (GPI-PLD) proteins and uses of these proteins, in particular in the treatment and diagnosis of diabetes and complications of diabetes such as insulin resistance, liver dysfunction, disorders involving pancreatectomies and conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock. The present invention further relates to variant GPI-PLD polypeptides.

Background of the Invention

Studies have shown that a number of cell surface proteins are attached to the cell membrane by covalent linkage to a glycosylphosphatidylinositol (GPI) anchor. It has been shown that the enzyme GPI-PLD cleaves the phosphodiester bond linking glycosylphosphatidylinositol to phosphatidic acid, thereby releasing anchored proteins.

GPI-PLD enzymes are abundantly present in human and bovine serum (5-10mg/ml in human serum). US Patent No: 5,418,147 (Huang et al) describes the purification of GPI-PLD from bovine liver, and the subsequent cloning of three GPI-PLD enzymes from bovine liver, human liver and human pancreas cDNA libraries. This patent reports the full length cDNA and amino acid sequences of the GPI-PLDs from human and bovine liver, and the partial cDNA and amino acid sequences of the human pancreatic form of the enzyme. Subsequently, the full length sequence of the pancreatic form of GPI-PLD was reported in Tsang et al (1992), and this enzyme has been found in cDNA libraries from breast, eye, spleen and tonsil. The three forms of

the enzymes are highly homologous with the predicted mature protein sequences of bovine liver GPI-PLD sharing 82% sequence identity with the human liver enzyme and 81% sequence identity with the human pancreatic enzyme. The amino acid sequences of human liver and pancreatic forms of GPI-PLD were deposited at GenBank under accession numbers L11701 and L11702 and consist of 841 and 840 amino acids respectively. The human liver and pancreatic forms of GPI-PLD share 94.6% sequence identity. The structure of GPI-PLDs is further discussed in Scallon et al, 1991.

However, despite cloning three forms of GPI-PLD, there is no suggestion in these references as to the *in vivo* role of the enzymes. Further, the only application of the enzymes suggested is in an expression system in which a heterologous protein is expressed in a host cell as a fusion with a GPI-signal peptide, leading to the heterologous protein becoming anchored to the cell membrane by a GPI anchor, where it can be cleaved off by coexpressed or added GPI-PLD.

GPI-PLD has also been isolated from human serum by Hoener et al (1992) and this form of the enzyme was found to be identical to the human pancreatic GPI-PLD apart from changes at 531 to 534 where VIGS is replaced by MLGT. This paper also showed that treatment of serum GPI-PLD with N-glycosidase F reduced the apparent molecular weight from 123 kD to 87 kD. Similarly, Li et al (1994) have shown that GPI-PLD was cleaved by trypsin into 3 fragments (2 x 40 kD and 30 kD), and Heller et al (1994) have shown that 33, 39 and 47kD species were produced, with only the N-terminal 39 kD fragment moiety showing enzyme activity after renaturation.

It has been proposed that one function of GPI-PLD enzyme is to produce inositolphosphoglycans (IPGs) by the cleavage of "free" GPIs in the plasma membrane in response to binding of a growth factor to its receptor (Rademacher et al, 1994). This role for GPI-PLD has been demonstrated in mast cells where IgE-dependent activation of these cells results in release of their granule contents, which include substances such as histamine, a mediator of the inflammatory response. In the presence of antigen, histamine is released; this release can be mimicked by addition of IPGs and is blocked by addition of anti-GPI-PLD antibodies (Lin et al, 1991).

The role of GPI-PLD in cleaving GPI-anchored proteins, and especially inositolphosphoglycans (IPGs), is examined in Jones et al (1997). However, the authors reflect the uncertainty in the art regarding the mechanism of IPG generation, noting that "The definitive activated enzyme, being a GPI-PLC or a GPI-PLD, has yet to be unequivocally identified" and that "little attention has been payed to the role of GPI-PLD as the hydrolysing enzyme".

In summary, despite the cloning of GPI-PLD enzymes and investigation as to their biochemical properties, the role of the enzyme *in vivo* or any possible medical use remains unknown.

Summary of the Invention

Broadly, the present invention relates to GPI-PLD for medical use, and in particular for the treatment of conditions which respond to GPI-PLD or which are characterised by reduced levels of active GPI-PLD in patients. The present invention relates in particular to the use of GPI-PLD in the treatment and diagnosis of diabetes and complications of diabetes, liver dysfunction

and disorders involving pancreatectomies, conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock. The GPI-PLD can be the forms of the enzyme disclosed in the prior art, or the GPI-PLDs disclosed for the first time here, including GPI-PLD variants which have a reduced susceptibility to phosphorylation by cAMP dependent protein kinase (PKA).

Accordingly, in first aspect, the present invention provides GPI-PLD for use in a method of medical treatment.

In a further aspect, the present invention provides a nucleic acid molecule encoding GPI-PLD for use in a method of medical treatment.

In a further aspect, the present invention provides the use of glycosylphosphatidylinositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of conditions that respond to GPI-PLD or which are characterised by reduced levels of active GPI-PLD as compared to a normal patient.

In a first embodiment, the present invention relates to the role of GPI-PLD in diabetes and diabetic complications.

Insulin is a major anabolic hormone and has both mitogenic and metabolic effects. Whilst much effort has been directed towards study of the cascade of intracellular phosphorylation events initiated by the binding of insulin to its cell surface receptor, the signalling arm mediated by IPGs has been largely overlooked. In one aspect, the present invention is

based on the realisation that GPI-PLDs are in fact the enzymes responsible for production of IPG second messengers following the binding of insulin to its receptor. The IPGs then interact with other cellular enzymes instigating some of the metabolic effects of the hormone. In particular, diabetic complications such as insulin resistance may be caused by deficiencies in GPI-PLD. Pancreatic islet cells produce and secrete GPI-PLD, which is transported in blood complexed with apolipoprotein A1, and may therefore represent the major source of circulating enzyme.

Insulin resistance is seen in both the early stages of type I (IDDM) and type II diabetes mellitus (NIDDM). If GPI-PLD levels are depleted by the destruction of pancreatic b-cells, as is seen in streptozotocin-treated rats, then this could be an important factor in the development of insulin resistance. This in turn suggests the treatment of such patients with GPI-PLD, optionally in combination with other diabetes therapies.

In a further aspect, the present invention provides the use of GPI-PLD for the preparation of a medicament for the treatment of diabetes, and in particular insulin dependent forms of diabetes.

In a further aspect, the present invention provides the use of GPI-PLD for the preparation of a medicament for the treatment of complications of diabetes, and in particular the treatment of insulin resistance.

In a further aspect, the present invention provides a method of treating a patient having diabetes or complications arising from diabetes, the method comprising administering to the patient a therapeutically

effective amount of GPI-PLD.

In all of the above aspects, GPI-PLD can be administered alone or in conjunction with other treatments for diabetes or diabetic complications, either sequentially or simultaneously.

In a further aspect, the present invention provides a kit comprising a composition including GPI-PLD and a second composition for the treatment of diabetes.

In a further aspect, the present invention provides the use of GPI-PLD levels or the levels of a product of GPI-PLD action, for example IPG or acyl-IPG, in the diagnosis of diabetes or diabetic complications. Thus, the present invention provides a method of diagnosing diabetes or diabetic complications, the method comprising determining the presence or amount of GPI-PLD or a product of GPI-PLD action in a biological sample from a patient. This determination can help in the diagnosis or prognosis of the patient, allowing the treatment of the patient to be tailored accordingly to the patient's individual needs.

In a second embodiment, the present invention relates to role of GPI-PLD in liver dysfunction and conditions involving pancreatectomies.

Thus, in a further aspect, the present invention provides the use of GPI-PLD for the preparation of a medicament for the treatment of liver dysfunction. Preferably, the GPI-PLD is administered in combination with apolipoprotein A1.

Treatment with GPI-PLD may also be applicable for

patients with pancreatectomies and disorders associated with this state, in which case it is preferably administered with apolipoprotein A1 or another suitable carrier such as a liposome.

In a further aspect, the present invention provides a method of treating a patient having liver dysfunction, the method comprising administering to the patient a therapeutically effective amount of GPI-PLD.

In all of the above aspects, GPI-PLD can be administered alone or in conjunction with other treatments for liver dysfunction, either sequentially or simultaneously.

In a further aspect, the present invention provides a kit comprising a composition including GPI-PLD, and optionally apolipoprotein A1, and a second composition for the treatment of liver dysfunction.

In a further aspect, the present invention provides a pharmaceutical composition comprising a nucleic acid molecule encoding a GPI-PLD protein and apolipoprotein A1.

In a further embodiment, the present invention relates to the role of GPI-PLD in conditions mediated by a product of an infectious organism, such as septic shock.

Thus, in a further aspect, the present invention provides the use of GPI-PLD in the treatment of conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD. The GPI-PLD can be of the forms of the enzyme disclosed in the prior art, or the GPI-PLDs disclosed for the first time here. An example of such a condition includes septic shock which commonly

occurs following abdominal surgery, severe burns, trauma or cardiac failure. Septic shock is generally preceded by a reduction in splanchnic blood flow, resulting in ischaemia and epithelial damage on reperfusion, allowing ingress of microorganisms and subsequent sepsis.

The present invention is based on the observation that in conditions such as septic shock, endotoxin is released from the microorganisms causing sepsis, leading to the clinical symptoms of septic shock such as total organ failure and fatal shock. The endotoxins can be glycolipids released from gram negative bacteria or glycolipids such as LAM released from *Mycobacteria* such as *Tuberculosis*. Without wishing to be bound by any particular theory, these endotoxins are believed to act by inhibiting GPI-PLD.

At present, despite many attempts in the art to develop a treatment for septic shock and other related conditions, there are no approved treatments available. In particular, a reliable diagnostic test for determining whether a patient has or is at risk of developing conditions such as septic shock would be useful as an early warning of the condition and to allow timely treatment to be given.

Accordingly, in a further aspect, the present invention provides the use of GPI-PLD for the preparation of a medicament for the treatment of a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD.

In a further aspect, the present invention provides a method of treating a patient having a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising

administering to the patient a therapeutically effective amount of GPI-PLD.

In the above aspects, the product of the infectious organism is typically an endotoxin, such as the glycolipids produced by gram negative or mycobacteria mentioned above.

In a further aspect, the present invention provides the use of GPI-PLD or IPG levels in the diagnosis of conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, and especially to the diagnosis of septic shock and/or distinguishing between different forms of septic shock. By way of example, the GPI-PLD or a product of GPI-PLD action can be determined by measuring the amount of the material and/or a characteristic activity of the material in the biological sample.

Thus, the present invention provides a method of diagnosing a condition mediated by a product of an infectious organism, the method comprising determining the presence or amount of GPI-PLD or a product of GPI-PLD action in a biological sample from a patient. This determination can help in the diagnosis or prognosis of the patient, allowing the treatment of the patient to be tailored accordingly to the patient's individual needs. IPGs or the acyl IPGs produced by GPI-PLD action can be used in this diagnosis as the inhibition of GPI-PLD by endotoxins is likely to cause the level of IPGs (e.g. in urine, blood etc) to drop since the GPI-PLD causes the release of IPG precursors. Thus, monitoring either or both of the level of GPI-PLD or the IPGs provides a way of assessing the likelihood of developing conditions such as septic shock or their prognosis. A determination of

the amount of GPI-PLD can be carried out using immobilised binding agents or by determining one or more of the activities associated with GPI-PLD and/or IPGs (see further below).

In a further general aspect, the present invention provides an expression vector comprising nucleic acid encoding GPI-PLD for use in a method of gene therapy, e.g. in the treatment of patients unable to produce sufficient GPI-PLD. The GPI-PLD encoding nucleic acid can be a sequence shown in Figures 4 to 6 or one of the known nucleic acid sequences.

In a further general aspect, the present invention provides a cell line for transplantation into a patient, wherein the cell line is transformed with nucleic acid encoding GPI-PLD, and is capable of expressing and secreting GPI-PLD. In one embodiment, the cell line is encapsulated, e.g. in a biocompatible polymer, so that the GPI-PLD produced by the cell line can be secreted into the patient, while preventing rejection by the immune system of the host. Methods for encapsulating cells in biocompatible polymers are described in WO93/16687 and WO96/31199.

In a further general aspect, the present invention provides a pharmaceutical composition comprising a nucleic acid molecule encoding a GPI-PLD protein.

In a further aspect, the present invention provides a pharmaceutical composition comprising a GPI-PLD protein.

The present invention also relates to novel GPI-PLD proteins and nucleic acid molecules, and in particular to forms of the protein having sequence differences compared

to the known human liver and pancreatic forms reported in the prior art.

In a further aspect, the present invention provides a substance which is an isolated polypeptide comprising a polypeptide having the amino acid sequence set out in Figure 3.

In a further aspect, the present invention provides isolated nucleic acid molecules encoding any one of the above polypeptides. Examples of such nucleic acid sequences are the nucleic acid sequences set out in Figures 4 to 6. The present invention also includes nucleic molecules having, for example, greater than 90% sequence identity with the nucleic acid sequences shown in these figures.

In further aspects, the present invention provides an expression vector comprising the above GPI-PLD proteins, nucleic acid operably linked to control sequences to direct its expression, and host cells transformed with the vectors. The present invention also includes a method of producing the above GPI-PLD proteins comprising culturing the host cells and isolating the GPI-PLD thus produced.

We have now also identified a phosphorylation site on GPI-PLD acted on by cAMP protein dependent kinase (PKA) which switches off the activity of the enzyme. This in turn makes it possible to make GPI-PLD variants having a reduced tendency to be phosphorylated, and consequently have an improved activity profile, and utility *in vitro* or *in vivo*.

Accordingly, the present invention provides variant GPI-

PLD polypeptides differing in amino acid sequence in the region corresponding to amino acid residues 689-692 (RRFS) of mature human wild-type GPI-PLD (corresponding to residues 713-716 of the sequence shown in Figure 7). These proteins have a reduced tendency or cannot be phosphorylated by the PKA (which is itself activated by the A-type IPGs released by GPI-PLD), and so are likely to have increased activity or half-life when used *in vitro* or *in vivo*.

Thus, present invention identifies for the first time a region between amino acids 689-692 which when modified, e.g. by a substitution, deletion or insertion of one or more amino acids, disrupts the phosphorylation site in this region. Preferred modifications are substitutions, and in particular substitutions to change the serine residue at position 692 to an amino acid other than serine or threonine.

Accordingly, in a first aspect, the present invention provides a variant GPI-PLD polypeptide comprising a modification within the motif RRFS present at amino acids 689 to 692 of wild-type mature human GPI-PLD.

In a further aspect, the present invention provides an isolated nucleic acid molecule encoding the variant GPI-PLD polypeptide.

In a further aspect, the present invention provides an expression vector comprising nucleic acid encoding a variant GPI-PLD polypeptide, operably linked to control sequences to direct its expression.

In further aspects, the present invention provides host cells transformed with said nucleic acid encoding a GPI-

PLD variant polypeptide, and methods of producing a variant GPI-PLD polypeptide comprising culturing the host cells so that the variant GPI-PLD polypeptide is expressed and isolating the polypeptide thus produced. The method may comprise the further step of then formulating the variant GPI-PLD polypeptide in a composition.

In a further aspect, the present invention provides the above variant GPI-PLD polypeptides or the nucleic acid molecules encoding them for use in methods of medical treatment, in particular the conditions described above.

In a further aspect, the present invention provides the use of a variant GPI-PLD polypeptide, or a nucleic acid molecule encoding it, for the preparation of a medicament for the treatment of conditions that respond to GPI-PLD.

These and other aspects of the present invention are described in more detail below.

By way of example, embodiments of the present invention will now be described in more detail with reference to the accompanying figures.

Brief Description of the Figures

Figure 1 shows an alignment of the deduced amino acid sequences of GPI-PLD encoded by cDNA clone A1 and the bovine and human liver GPI-PLD sequences disclosed in US Patent No: 5,418,147 (Huang et al).

Figure 2 shows the nucleic acid sequence from cDNA clone A1 aligned with the pancreatic forms of GPI-PLD disclosed in US Patent No: 5,418,147 (Huang et al) (partial sequence) and the corresponding full length nucleic acid

sequence deposited at GenBank.

Figure 3 shows the amino acid sequences of the GPI-PLDs in clones a1, b2 and d3, and consist of 840, 795 and 510 amino acids respectively.

Figure 4 shows the nucleic acid sequence of cDNA clone a1 encoding GPI-PLD, consisting of 2832 bp.

Figure 5 shows the nucleic acid sequence of cDNA clone b2 encoding GPI-PLD, consisting of 2472 bp.

Figure 6 shows the nucleic acid sequence of cDNA clone d3 encoding GPI-PLD, consisting of 1942 bp.

Figure 7 shows an alignment of the deduced amino acid sequences of GPI-PLDs encoded by cDNA clones a1, b2 and d3 with the pancreatic form of the enzyme deposited at GenBank under accession number 11702.

Figure 8 shows an alignment of the nucleic acid sequences from cDNA clones a1, b2 and d3 with the cDNA sequence encoding the human pancreatic form of GPI-PLD deposited at GenBank under accession number L11702.

Detailed Description

GPI-PLD Proteins

The term "GPI-PLD biological activity" is herein defined as the enzymatic activity of GPI-PLD in cleaving the photodiester bond linking glycosylphosphatidylinositol to phosphatidic acid, e.g. releasing a GPI-anchored protein. As noted in Heller et al (1994), this activity has been localised to the N-terminal 39 kD portion of full length GPI-PLD.

The medical uses of GPI-PLD described herein can use the

novel GPI-PLD variants or the forms of the enzyme disclosed in the prior art. In either event, the skilled person can use the techniques described herein and others well known in the art to produce large amounts of these proteins, or fragments or active portions thereof, for use as pharmaceuticals, in the developments of drugs and for further study into its properties and role *in vivo*.

In a further aspect of the present invention provides a polypeptide having the amino acid sequence shown in Figure 3, which may be in isolated and/or purified form, free or substantially free of material with which it is naturally associated. In one embodiment, the clone a1 has an amino acid sequence consisting of 840 amino acids, a 23 amino acid signal peptide and a 817 amino acid mature protein. The present invention relates to both GPI-PLD proteins (and variants thereof) with and without the signal peptide, i.e. comprising amino acids 1-840 or 24-840 as shown in the figures.

GPI-PLD proteins which are amino acid sequence variants or alleles can also be used in the present invention. A polypeptide which is a variant or allele may have an amino acid sequence which differs from that given in Figures 1 or 3 by one or more of addition, substitution, deletion and insertion of one or more amino acids. Preferred polypeptides have GPI-PLD enzymatic function as defined above.

A GPI-PLD protein which is an amino acid sequence variant or allele of an amino acid sequence shown in Figures 1 or 3 may comprise an amino acid sequence which shares greater than about 70%, greater than about 80%, greater than about 90%, greater than about 95%, greater than about 97%, greater than about 98% or greater than about

99% sequence identity with an amino acid sequence shown in Figures 1 or 3. Sequence comparison and identity calculations were carried out using the Cluster program (Thompson et al, 1994), using the following parameters (Pairwise Alignment Parameters: Weight Matrix: pam series; Gap Open Penalty: 10.00; Gap Extension Penalty: 0.10). Alternatively, the GCG program could be used which is available from Genetics Computer Group, Oxford Molecular Group, Madison, Wisconsin, USA, Version 9.1. Particular amino acid sequence variants may differ from those shown in Figures 1 and 3 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20 20-30, 30-50, 50-100, 100-150, or more than 150 amino acids.

The variant GPI-PLD polypeptides of the present invention differ in amino acid sequence as compared to human GPI-PLD at the phosphorylation site from amino acids 689 to 692 of the mature sequence (corresponding to amino acids 713-716 shown in Figure 7), i.e. within the amino acid motif RRFS. The term 'variant GPI-PLD polypeptide' is intended, inter alia, to include polypeptides which are modified within this region by deletion, substitution and/or insertion of one or more amino acids. These sequence differences may be the result of varying the GPI-PLD amino acid sequence of a parent GPI-PLD polypeptide, either a wild type GPI-PLD polypeptide or a GPI-PLD polypeptide comprising one or more other modifications, e.g. by manipulation of the nucleic acid encoding the polypeptide, by altering the polypeptide itself or by the *de novo* synthesis of the variant protein. In preferred embodiments, the GPI-PLD retains, at least in part, one of its biological activities, e.g. by the presence of a functional N-terminal domain.

A deletion may take the form of the deletion of one, two, three or all four amino acids within the region. In some embodiments, the deletion may be part of a larger deletion encompassing a greater part of the GPI-PLD molecule. In a preferred embodiment, the variant GPI-PLD polypeptides have an amino acid sequence which differs from the amino acid sequence of human wild type GPI-PLD by the deletion comprising residues 689 to 692 inclusive.

An insertion may take the form of 1, 2, 3, 4 or 5 or more additional amino acids inserted between amino acids within the RRFS motif to disrupt it.

A substitution may take the form of the substitution of one, two, three or all of the four amino acids within the region corresponding to amino acids 689 to 692 of wild type human GPI-PLD. The substitutions within this region may be part of a more extensive series of substitutions encompassing other parts of the GPI-PLD polypeptide. In particular, mutant forms of GPI-PLD which may have practical use differ from the wild type sequence. Some of these mutants are used in the experiments described below.

In all cases, it is preferred that the resulting GPI-PLD variant retains or has an increased GPI-PLD biological activity as compared to human wild type GPI-PLD, and more especially the enzymatic activity of GPI-PLD in cleaving the photodiester bond linking GPI to phosphatidic acid, and thereby releasing a GPI-anchored protein.

The present invention also includes the use of active portions and fragments of the GPI-PLD proteins.

An "active portion" of GPI-PLD protein is a polypeptide

which is less than said full length GPI-PLD protein, but which retains at least one its essential biological activity, e.g. the enzyme activity mentioned above known to be located in the N-terminal 39kD portion of the enzyme. For instance, portions of GPI-PLD protein can act as sequestrators or competitive antagonists by interacting with other proteins.

A "fragment" of the GPI-PLD protein means a stretch of amino acid residues of at least about 5 to 7 contiguous amino acids, often at least about 7 to 9 contiguous amino acids, typically at least about 9 to 13 contiguous amino acids, more preferably at least about 20 to 30 or more contiguous amino acids, more preferably greater than 40 amino acids, more preferably greater than 100 amino acids.

A polypeptide according to the present invention may be isolated and/or purified (e.g. using an antibody) for instance after production by expression from encoding nucleic acid (for which see below). Polypeptides according to the present invention may also be generated wholly or partly by chemical synthesis. The isolated and/or purified polypeptide may be used in formulation of a composition, which may include at least one additional component, for example a pharmaceutical composition including a pharmaceutically acceptable excipient, vehicle or carrier. A composition including a polypeptide according to the invention may be used in prophylactic and/or therapeutic treatment as discussed below.

The GPI-PLD polypeptides can also be linked to a coupling partner, e.g. an effector molecule, a label, a drug, a toxin and/or a carrier or transport molecule. Techniques

for coupling the peptides of the invention to both peptidyl and non-peptidyl coupling partners are well known in the art. In one embodiment, the carrier molecule is a 16 aa peptide sequence derived from the homeodomain of *Antennapedia* (e.g. as sold under the name "Penetratin"), which can be coupled to a peptide via a terminal Cys residue. The "Penetratin" molecule and its properties are described in WO91/18981.

A and P-type IPGs

Studies have shown that A-type mediators modulate the activity of a number of insulin-dependent enzymes such as cAMP dependent protein kinase (inhibits), adenylate cyclase (inhibits) and cAMP phospho-diesterases (stimulates). In contrast, P-type mediators modulate the activity of insulin-dependent enzymes such as pyruvate dehydrogenase phosphatase (stimulates), glycogen synthase phosphatase (stimulates), and cAMP dependent protein kinase (inhibits). The A-type mediators mimic the lipogenic activity of insulin on adipocytes, whereas the P-type mediators mimic the glycogenic activity of insulin on muscle. Both A-and P-type mediators inhibit cAMP dependent protein kinase and are mitogenic when added to fibroblasts in serum free media. The ability of the mediators to stimulate fibroblast proliferation is enhanced if the cells are transfected with the EGF-receptor. A-type mediators can stimulate cell proliferation in the chick cochleovestibular ganglia.

Soluble IPG fractions having A-type and P-type activity have been obtained from a variety of animal tissues including rat tissues (liver, kidney, muscle brain, adipose, heart) and bovine liver. A-type and P-type IPG biological activity has also been detected in human liver and placenta, malaria parasitized RBC and mycobacteria.

The ability of an anti-inositolglycan antibody to inhibit insulin action on human placental cytotrophoblasts and BC3H1 myocytes or bovine-derived IPG action on rat diaphragm and chick cochleovestibular ganglia suggests cross-species conservation of many structural features.

A-type substances are cyclitol-containing carbohydrates, also containing Zn^{2+} ions and optionally phosphate and having the properties of regulating lipogenic activity and inhibiting cAMP dependent protein kinase. They may also inhibit adenylate cyclase, be mitogenic when added to EGF-transfected fibroblasts in serum free medium. A-type IPGs isolated from sources such as human or bovine liver have the property of stimulating lipogenesis in adipocytes. In contrast, the A-type substances from porcine tissue have the properties of inhibiting lipogenesis and lowering blood glucose levels when administered to diabetics, i.e. patients or a suitable animal model.

P-type substances are cyclitol-containing carbohydrates, also containing Mn^{2+} and/or Zn^{2+} ions and optionally phosphate and having the properties of regulating glycogen metabolism and activating pyruvate dehydrogenase phosphatase. They may also stimulate the activity of glycogen synthase phosphatase, be mitogenic when added to fibroblasts in serum free medium, and inhibit cAMP dependent protein kinase.

Methods for obtaining A-type and P-type IPGs are set out in Caro et al, 1997, and in WO98/11116 and WO98/11117.

Pharmaceutical Compositions

As mentioned above, GPI-PLD proteins including the variant proteins can used for treating diabetes and the

complications of diabetes (e.g. insulin resistance), optionally in conjunction with other treatments for these disorders.

GPI-PLD proteins can be administered alone or in combination with other treatments for diabetes or diabetic complications, either simultaneously or sequentially. Examples of known diabetes treatments include (a) insulin, which is typically delivered by injection, (b) oral insulin compositions, (c) glucose sparing or insulin enhancing drugs, (d) a-glucosidase inhibitors to reduce carbohydrate absorption (precose and miglitol), and (e) drugs used to treat patients with insulin sensitivity, e.g. thiazolidinediones, such as Rezulin, rosiglitazone, piogliazone and tyrosine phosphatase inhibitors.

In further embodiments, the GPI-PLD can be administered with P and/or A-type IPGs, and/or antagonists of these substances. Methods for obtaining A-type and P-type IPGs and their antagonists are set out in Caro et al, 1997, and in WO98/11116 and WO98/11117.

The role of P and A-type IPGs and their use in the diagnosis and treatment of diabetes is disclosed in WO98/11435. In summary, this application discloses that in some forms of diabetes the ratio of P:A type IPGs is imbalanced and can be corrected by administering a medicament comprising the appropriate ratio of P or A-type IPGs or antagonist thereof. In particular, WO98/11435 describes the treatment of obese type II diabetes (NIDDM) patients with a P-type IPG or with an A-type IPG antagonist, such as antibodies which bind specifically to the A-type IPG, and the treatment of IDDM or lean type II diabetes (NIDDM) (body mass index < 27)

with a mixture of A and P-type IPGs, typically in a P:A ratio of about 6:1 for males and 4:1 for females.

The compositions of the invention can be used in the treatment of diabetes, in particular insulin dependent forms of diabetes (type I and type II diabetes). They can also be used in the treatment of the complications of diabetes and in particular forms of insulin resistance such as insulin resistance in type I or type II diabetes and brittle diabetes.

In a further aspect, GPI-PLD proteins can used for treating liver dysfunction, optionally in conjunction with other treatments for these disorders. Preferably, the GPI-PLD is administered with apolipoprotein A1, and more preferably, as a complex with this substance. The isolation of apolipoprotein A1 is described in Hoener et al (1993), Deeg et al (1994) and Brewer et al (1986). The compositions can be used to treat liver dysfunction conditions which are characterised by reduced levels of apolipoprotein A1 and/or GPI-PLD and/or apolipoprotein A1/GPI-PLD complex.

GPI-PLD proteins can be administered alone or in combination with other treatments for liver dysfunction, either simultaneously or sequentially.

In a further aspect, GPI-PLD proteins and IPGs can used for treating treatment of conditions caused by a product of an infectious organism which is capable of inhibiting GPI-PLD.

As mentioned above, in further embodiments, the GPI-PLD can be administered alone or in combination with P and/or A-type IPGs.

In all of the above embodiments, the GPI-PLD proteins and any accompanying compositions can be formulated in pharmaceutical compositions, which may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as sodium chloride injection, Ringer's injection, lactated Ringer's injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included as required.

Whether it is a polypeptide, peptide, nucleic acid molecule, small molecule or other pharmaceutically useful compound of the invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

GPI-PLD nucleic acid

"GPI-PLD nucleic acid" includes a nucleic acid molecule which has a nucleotide sequence encoding a polypeptide which includes the amino acid sequence shown in Figures 4 to 6, and in some embodiments of the invention extends to the known human liver and pancreatic forms of GPI-PLD (L11701 and L11702). These forms of GPI-PLD have been mapped to human chromosome 6 and are contained in the 4 centimorgan region of D6S1660-D6S1558 at positions 95.95 and 99.71 (NCBI GeneMap'98). The gene starts in the cytogenic region corresponding to 6p22.3 and extends into 6p21.3. This region also contains the IDDM1 and HLA loci (although the HLA genes map to the adjacent D6S1558-D6S1616 interval). The mouse GPI-PLD gene has also been

mapped to chromosome 13, near the *fim 1* locus, which is found in humans on chromosome 6.

The GPI-PLD coding sequence may be that shown in Figures 2, 4 to 6 or 8, a complementary nucleic acid sequence, or it may be a mutant, variant, derivative or allele of these sequences. The sequence may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

The encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in the figures. Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant or allele of the sequence shown in Figures 1, 3 or 7 is further provided by the present invention. Such polypeptides are discussed below. Nucleic acid encoding such a polypeptide may show greater than about 70% identity, greater than about 80% identity, greater than about 90% identity, greater than about 95% identity, greater than about 98% identity, or greater than about 99% identity with a sequence shown in the figures.

The present invention also includes fragments of the GPI-PLD nucleic acid sequences described herein, the fragments preferably being at least 12, 15, 30, 45, 60, 120 or 240 nucleotides in length.

Generally, nucleic acid according to the present invention is provided as an isolate, in isolated and/or purified form, or free or substantially free of material

with which it is naturally associated, such as free or substantially free of nucleic acid flanking the gene in the human genome, except possibly one or more regulatory sequence(s) for expression. Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

Nucleic acid sequences encoding all or part of the GPI-PLD gene and/or its regulatory elements can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook, Fritsch and Maniatis, "Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989, and Ausubel et al, Short Protocols in Molecular Biology, John Wiley and Sons, 1992). These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic sources, (ii) chemical synthesis, or (iii) amplification in *E. coli*. Modifications to the GPI-PLD sequences can be made, e.g. using site directed mutagenesis, to provide expression of modified GPI-PLD protein or to take account of codon preference in the host cells used to express the nucleic acid.

In order to obtain expression of the GPI-PLD nucleic acid sequences, the sequences can be incorporated in a vector having control sequences operably linked to the GPI-PLD nucleic acid to control its expression. The use of expression systems has reached an advanced degree of sophistication. The vectors may include other sequences such as promoters or enhancers to drive the expression of

the inserted nucleic acid, nucleic acid sequences so that the GPI-PLD protein is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide produced in the host cell is secreted from the cell. GPI-PLD protein can then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the GPI-PLD protein is produced and recovering the GPI-PLD protein from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of *E. coli*, yeast, and eukaryotic cells such as COS or CHO cells. The choice of host cell can be used to control the properties of the GPI-PLD protein expressed in those cells, e.g. controlling where the polypeptide is deposited in the host cells or affecting properties such as its glycosylation and phosphorylation.

PCR techniques for the amplification of nucleic acid are described in US Patent No:4,683,195. In general, such techniques require that sequence information from the ends of the target sequence is known to allow suitable forward and reverse oligonucleotide primers to be designed to be identical or similar to the polynucleotide sequence that is the target for the amplification. PCR comprises steps of denaturation of template nucleic acid (if double-stranded), annealing of primer to target, and polymerisation. The nucleic acid probed or used as template in the amplification reaction may be genomic DNA, cDNA or RNA. PCR can be used to amplify specific sequences from genomic DNA, specific RNA sequences and cDNA transcribed from mRNA, bacteriophage or plasmid sequences. The GPI-PLD protein nucleic acid sequences provided herein readily allow the skilled person to design PCR primers. References for the general use of

PCR techniques include Mullis et al, Cold Spring Harbor Symp. Quant. Biol., 51:263, 1987; Ehrlich (ed), PCR Technology, Stockton Press, NY, 1989; Ehrlich et al, Science, 252:1643-1650, 1991; "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, Academic Press, New York, 1990.

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridize with one or more fragments of the nucleic acid sequence shown in the figures, particularly fragments of relatively rare sequence, based on codon usage or statistical analysis. A primer designed to hybridize with a fragment of the nucleic acid sequence shown in the above figures may be used in conjunction with one or more oligonucleotides designed to hybridize to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE" (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an oligonucleotide linker and PCR is performed using a primer which hybridizes with a GPI-PLD nucleic acid sequence shown in figures and a primer which hybridizes to the oligonucleotide linker.

Such oligonucleotide probes or primers, as well as the full-length sequence (and mutants, alleles, variants and derivatives) are also useful in screening a test sample containing nucleic acid for the presence of alleles, mutants and variants, especially those that lead to the production of inactive forms of GPI-PLD protein, the probes hybridizing with a target sequence from a sample obtained from the individual being tested. The conditions of the hybridization can be controlled to minimise non-specific binding, and preferably stringent to moderately stringent hybridization conditions are

preferred. The skilled person is readily able to design such probes, label them and devise suitable conditions for the hybridization reactions, assisted by textbooks such as Sambrook et al (1989) and Ausubel et al (1992).

Examples of "stringent conditions" are those which: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulphate at 50°C; (2) employ during hybridisation a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% BSA/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750mM sodium chloride, 75mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50mg/ml), 0.1% SDS, and 10% dextran sulphate at 42°C, with washes at 42°C in 0.2 x SSC and 50% formamide at 55°C, followed by high stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. These hybridisation conditions may be used in the context of defining nucleic acid sequences which hybridize with GPI-PLD nucleic acid sequences.

Uses of GPI-PLD Nucleic Acid

The GPI-PLD nucleic acid sequences can be used in the preparation of cell lines capable of expressing GPI-PLD and included in expression vectors or otherwise formulated, e.g. for use in gene therapy techniques.

Thus, the present invention provides a cell line for transplantation into a patient, the cell line being transformed with nucleic acid encoding GPI-PLD, and being capable of expressing and secreting GPI-PLD. In one

embodiment, the cell lines are encapsulated, e.g. in a biocompatible polymer, so that the GPI-PLD produced by the cell line can be secreted into the patient, while preventing rejection by the immune system of the host. Methods for encapsulating cells in biocompatible polymers are described in WO93/16687 and WO96/31199.

As a further alternative, the nucleic acid encoded the GPI-PLD protein could be used in a method of gene therapy, to treat a patient who is unable to synthesize the active polypeptide or unable to synthesize it at the normal level, thereby providing the effect provided by wild-type GPI-PLD protein and suppressing the occurrence of diabetes in the target cells.

Vectors such as viral vectors have been used in the prior art to introduce genes into a wide variety of different target cells. Typically, the vectors are exposed to the target cells so that transfection can take place in a sufficient proportion of the cells to provide a useful therapeutic or prophylactic effect from the expression of the desired polypeptide. The transfected nucleic acid may be permanently incorporated into the genome of each of the targeted tumour cells, providing long lasting effect, or alternatively the treatment may have to be repeated periodically.

A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see US Patent No: 5,252,479 and WO93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses.

As an alternative to the use of viral vectors other known methods of introducing nucleic acid into cells includes electroporation, calcium phosphate co-precipitation, mechanical techniques such as microinjection, transfer mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer.

As mentioned above, the aim of gene therapy using nucleic acid encoding the GPI-PLD protein, or an active portion thereof, is to increase the amount of the expression product of the nucleic acid in cells in which the level of the wild-type GPI-PLD protein is absent or present only at reduced levels. Target cells for gene therapy include insulin secreting b-cells or any neuron derived cells. Cell engineering can be used to provide the overexpression or repression of GPI-PLD protein in transfected cell lines which can then be subsequently transplanted to humans. Gene therapy can be employed using a promoter to drive GPI-PLD protein expression in a tissue specific manner (i.e. an insulin promoter linked to GPI-PLD cDNA will overexpress GPI-PLD protein in b-cells and transiently in the brain). If defective function of GPI-PLD protein is involved in neurological disease, GPI-PLD protein can be overexpressed in transformed cell lines for transplantation.

Gene transfer techniques which selectively target the GPI-PLD nucleic acid to target tissues are preferred. Examples of this included receptor-mediated gene transfer, in which the nucleic acid is linked to a protein ligand via polylysine, with the ligand being specific for a receptor present on the surface of the target cells.

Diagnostic Methods

Methods for determining the concentration of analytes in biological samples from individuals are well known in the art and can be employed in the context of the present invention to determine the presence or amount of GPI-PLD or a product of GPI-PLD action in a biological sample from a patient. This in turn can allow a physician to determine whether a patient suffers from one of the conditions discussed above and so optimise the treatment of it.

As discussed above, the conditions include diabetes and diabetic complications, liver dysfunction or disorders involving pancreatectomies, and conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock.

Broadly, the methods divide into those screening for the presence of GPI-PLD protein nucleic acid sequences and those that rely on detecting the presence or absence of the GPI-PLD protein polypeptide or a product of GPI-PLD action (e.g. IPGs or acyl-IPGs). The methods make use of biological samples from individuals that are suspected of contain the nucleic acid sequences or polypeptide.

These diagnostic methods can employ biological samples such as blood, serum, tissue samples or urine. In view of the fact that the activity of GPI-PLD is thought to be due to the level of the enzyme circulating in serum, the use of serum or blood samples is preferred.

The assay methods for determining the amount or concentration of GPI-PLD protein typically either employ binding agents having binding sites capable of specifically binding to GPI-PLD or the product of GPI-PLD action in preference to other molecules or measure a

characteristic biological activity of GPI-PLD. Examples of binding agents include antibodies, receptors and other molecules capable of specifically binding the enzyme. Conveniently, the binding agent(s) are immobilised on solid support, e.g. at defined locations, to make them easy to manipulate during the assay.

In one format, the methods of diagnosing the conditions relating to GPI-PLD disclosed herein comprises the steps of:

(a) contacting a biological sample obtained from the patient with a solid support having immobilised thereon a binding agent having binding sites specific for GPI-PLD or a product of GPI-PLD action;

(b) contacting the solid support with one or more labelled developing agents capable of binding to unoccupied binding sites, bound GPI-PLD or product, or occupied binding sites; and,

(c) detecting the label of the developing agents specifically binding in step (b) to obtain a value representative of the amount of GPI-PLD or the product of GPI-PLD action in the sample.

Alternatively or additionally, the method can assess GPI-PLD levels by measuring one of its biological activities, which are discussed further below.

The products of GPI-PLD action include acyl-IPGs and IPGs, the characteristic activities of which are discussed above. Antibodies which are capable of binding to IPGs are disclosed in WO98/1116, WO98/11117 and WO99/47565.

The sample is generally contacted with the binding agent(s) under appropriate conditions so that GPI-PLD

present in the sample can bind to the binding agent(s). The fractional occupancy of the binding sites of the binding agent(s) can then be determined using a developing agent or agents. Typically, the developing agents are labelled (e.g. with radioactive, fluorescent or enzyme labels) so that they can be detected using techniques well known in the art. Thus, radioactive labels can be detected using a scintillation counter or other radiation counting device, fluorescent labels using a laser and confocal microscope, and enzyme labels by the action of an enzyme label on a substrate, typically to produce a colour change. The developing agent(s) can be used in a competitive method in which the developing agent competes with the analyte for occupied binding sites of the binding agent, or non-competitive method, in which the labelled developing agent binds analyte bound by the binding agent or to occupied binding sites. Both methods provide an indication of the number of the binding sites occupied by the analyte, and hence the concentration of the analyte in the sample, e.g. by comparison with standards obtained using samples containing known concentrations of the analyte.

Experimental

In one embodiment, the present invention is based on the realisation that GPI-PLD is responsible for the production of IPG second messengers following binding of insulin to its receptor. The IPGs then interact with other cellular enzymes instigating some of the metabolic effects of the hormone. In view of this, insulin resistance may be caused by deficiencies in GPI-PLD; it has shown that pancreatic islet cells produce and secrete GPI-PLD, which is transported in blood complexed with apolipoprotein A1, and may therefore represent the major source of circulating enzyme. If this is indeed the case

then the insulin resistance seen in early type I diabetes mellitus (IDDM) may result from decreased circulating GPI-PLD levels. This may have direct therapeutic relevance in that co-infusion of insulin with GPI-PLD may in fact be a far more effective therapy for diabetic patients than insulin.

In a further embodiment, the present invention is based on the realisation that GPI-PLD can be used in the treatment of liver dysfunction, and in particular combination with apoliprotein A1 to which it is bound in human serum and blood. As GPI-PLD is transported in blood complexed with apolipoprotein A1, liver dysfunction, and especially dysfunction characterised by reduced apolipoprotein A1 levels, can be treated using GPI-PLD.

In a third embodiment, the present invention is based on the observation that in conditions such as septic shock, endotoxin is released from the microorganisms causing sepsis, leading to the clinical symptoms of septic shock such as total organ failure and fatal shock. The endotoxins can be glycolipids released from gram negative bacteria or glycolipids such as LAM released from *Mycobacteria* such as *Tuberculosis*. Without wishing to be bound by any particular theory, these endotoxins are believed to act by inhibiting GPI-PLD.

Screening of human liver cDNA library

A human liver cDNA library (Gibco BRL, cat # 10422-012, lot # HF4703) was screened for GPI-PLD, resulting in the isolation of 3 cDNA clones. The nucleic acid sequences of the clones are shown in Figures 4 to 6, with the deduced amino acid sequences shown in Figure 3.

Clone a1 represents the full length cDNA. There are only two differences within the coding region of this sequence when compared to that of the human GPI-PLD pancreatic form described in the GenBank database (accession number L11702). These are *a g* to *a* conversion at positions 88 (L11702), 199 (a1) and a *t* to *g* conversion at positions 797 (L11702), 908(a1). Interestingly this latter conversion creates a unique *HindIII* restriction site in the a1 clone. Both conversions result in amino acid differences, the first changes amino acid 30 from a valine in L11702 to an isoleucine in a1, and the second changes amino acid 266 from an isoleucine in L11702 to a serine in a1. Clone a1 also differs from L11702 in that it contains 5' untranslated region (UTR) and only shares the first 168 bases of the 3' UTR before terminating in a poly-A tail.

Clone b2 lacks exons 23-25 of GPI-PLD, which begins at position 2469 in the a1 nucleotide sequence. However, the sequence from here to the end of b2 (2444-2473) does not contain a stop codon. It is therefore not clear whether b2 represents a cDNA with a different final exon or is the produce of aberrant processing.

Clone d3 shared the 3'coding and 3'UTR sequence of the a1 clone from position 1119 onwards, however the initial 1008 base pairs of coding sequence representing the initial 12 exons, are absent from this clone. Clone d3 contains a methionine initiation codon in frame to the coding sequence at position 202 and a unique 5' UTR. Translation of d3 from this codon would result in a unique sequence of 6 amino acids (1-6). Clone d3 therefore appears to represent a true transcript, in that it contains initiation and stop codons and both 5' and 3' UTRs. The predicted protein product of this transcript

would apparently lack the catalytic domain, which has been localised to the N-terminus of the GPI-PLD enzyme (amino acids 1-375), however the 4 EF hand-like domains would still be present.

Huang et al and Tsang et al (1992) reported that two variants or isoenzymes of GPI-PLD exist, the so-called liver and pancreatic forms (accession numbers L11701 and 11702). Other workers have detected L11702 cDNAs in human breast, eye, spleen, tonsil, and pancreas, as well as in liver. However, we failed to detect the liver form of GPI-PLD in the liver or in any other tissues.

Gene mapping and localisation

The chromosomal gene isolated in the experiments above is over 100 kb in length. The gene was also isolated on a PAC and mapped by fluorescence-in situ hybridisation (FISH) to 6p22.3 extending into 6p21.3, agreeing with recent radiation hybrid maps as seen on GeneMap'98; NCBI). The IDDM1 susceptibility gene also maps to 6p21.3, although recent evidence suggests that at least two closely-linked loci for IDDM1 are in the MHC region. The MHC locus itself seems to map to a region adjoining the GPI-PLD locus rather than within the same microsatellite band, so the significance of the proximity of the GPI-PLD and IDDM1 loci is unclear.

Northern blots of the mRNA species found in liver have shown two presumed splice variants as well as the full-length transcript. One has a deletion of about 160 amino acids from the mature 817 amino acid protein. The other seems to be a C-terminal deletion, which may therefore be non-functional if other authors are correct in finding that the C-terminus is necessary for enzyme activity.

The predominant GPI-PLD species detected after tissue extraction by antibodies (Western blots) has apparent molecular weight of about 47 kD, which agrees with other authors that full-length GPI-PLD is taken up from the plasma and processed to smaller active species.

PCR Analysis of GPI-PLD isoforms

PCR was used to compare the expression of putative cDNAs L11701 and L11702 using oligos pairs in cDNA made from human liver mRNA or in genomic DNA. cDNA synthesis reactions from which reverse transcriptase was omitted served as negative controls.

Two regions of the cDNAs were found to have a sufficient number of base differences to enable the synthesis of isoform-specific oligonucleotides. Region 1 contained 6 base pair changes over a total length of 25 nucleotides. From this region two isoform-specific reverse oligos were made:

P2	cagc**a**g**a**ggctgc**gc**gtc**a**gatatg	(L11702: 2115-2091)
L2	cagc**gg**t**g**gctgc**ag**gtc**g**gatg**t**g	(L11701: 2150-2126)

These were matched with forward oligos for gc content from a region approximately 700bp upstream. This region is shown below with differences highlighted in bold and the oligo sequences underlined:

P1 **g**tgttg**g**actttaac**g**t**g**ga**c**ggc**g**tgcctgacctggccg
(L11702: 1366-1405)
L1 **a**tgttggactttaac**a**t**g**ga**t**ggc**g**tgcctgacctggccg
(L11701: 1400-1440)

Region 2 (1 (L11701; L11702) contained 9 base pair

changes over a total length of 32 nucleotides and was used to make two isoform-specific reverse oligos as before:

P19 **gtac**gta**g**gggctcc**a**accagcagcac**t**tg**tt**(L11702: 2019-1988)

L4 **acgt**gtc**g**gggctccc**a**ccagcagcac**c**tg**gg**(L11701: 2054-2023)

These oligos were paired with a single oligo which recognizes both isoforms approximately 300bp upstream which would also enable PCR from genomic DNA:

U2 tggttgggagcccgacctggaagaatgccagc
(L11702: 1787-1818; L11701: 1822-1853)

5mg total human liver RNA (Invitrogen) was reverse transcribed using Superscript II (GibcoBRL) for 90 mins in a total volume of 35ul. Negative controls contained 5mg of RNA but no reverse transcriptase (lanes 2, 4, 6 and 9). 2.5ml of this reaction or 888ng of human genomic DNA (Promega) was transferred to a 50ml PCR reaction containing 25pmoles of each oligo. After an initial 4 min 94°C denaturing cycle, 30 cycles were performed (25 secs denaturing - 94°C, 30 secs annealing, 30 secs extension - 72°C) and PCR products resolved on a 1% agarose gel. Annealing temperatures of the oligo pairs were as follows: P1 & P2 - 62°C; L1 & L2 - 66°C; U2 & P19 - 68.3°C; U2 & L4 - 71.5°C).

Southern Blot

A Southern blot of PAC 282J10 DNA and human genomic DNA was hybridised with a cDNA probe containing exons 15-19. The same bands hybridise in both PAC and genomic DNA therefore suggesting that only one copy of the GPI-PLD gene is present in the human genome. This result is in accord with the finding of only one gene in the mouse.

(LeBoeuf et al, Mammalian Genome 9:710-714, 1998).

4mg of human genomic DNA or 1mg of PAC 282J10 DNA was digested with the restriction enzymes ApaI, EcoRI or NsiI (Promega) at 37°C overnight and run on 1% agarose gel, which was denatured, neutralised and blotted in 20XSSC overnight. DNA was UV crosslinked onto the blot and then hybridised with ^{32}P-labelled P1/P2 PCR product. The blot was then washed with decreasing SSC concentrations, the final wash being 0.2XSSC, 0.1%SDS for 20 mins at 65°C. Autoradiographs were exposed at -80°C for 1h (282J10) or 3 days (genomic).

GPI-PLD gene structure

The structure of the human GPI-PLD gene has been determined. It comprises 25 exons and extends over more than 100 kb of chromosome 6p22.3 into 6p21.3. We have used Southern blot analysis to determine that only one GPI-PLD gene exists in the human genome.

Using PCR analysis as described above, we have been unable to prove the existence of the so-called liver form of GPI-PLD (GenBank accession number L11701), whereas the so called pancreas form (L11702) is the form we have detected in human liver. These data show that the two forms do not exist alongside each other in the human liver, however it is still possible that L11701 represents a polymorphic variant not seen in the subjects from whom our liver RNA was obtained.

GPI-PLD gene expression

Using PCR we have compared the expression of GPI-PLD in cDNA libraries made from human tissues. GPI-PLD appears most abundant in the liver followed by the lung. A very low level of expression was seen in kidney and heart and

skeletal muscle, however we were unable to detect expression in pancreas, brain or placenta.

Recombinant GPI-PLD has been purified from stable CHO cell lines transfected with the full-length human GPI-PLD cDNA clone a1 isolated previously from a human liver cDNA library. Recombinant GPI-PLD cleaves the GPI substrate mfVSG, and like its counterpart purified from serum, this action is inhibited by prior incubation with the transition metal ion chelator 1,10-phenanthroline.

We have identified at least two systems which do not appear to express the GPI-PLD gene, namely the human placenta and the rat basophil-like cell line RBL2H3. However in both cases abundant GPI-PLD protein and enzyme activity is detectable, thus confirming our prediction that in tissues which do not express the gene, protein is still expressed and is presumably uptaken from the vast reserves found in serum. Experiments using the mouse skeletal muscle cell line C2C12 indicate that over 70% of the GPI-PLD activity present within the cells is derived from serum.

GPI-PLD obtained from serum by cells is required for second messenger signalling

The principle goal of these experiments was to determine the role of glycosylphosphatidylinositol phospholipase D (GPI-PLD) in a type one hypersensitivity reaction. This reaction involved the cross-linking of IgE receptors on the mast cell surface, leading to the release of allergic mediators.

Such an allergic reaction has been experimentally reproduced in our laboratory, using a rat basophilic leukaemia cell line, RBL-2H3. These cells naturally have

unoccupied IgE receptors (FceR1, or high-affinity receptors), allowing them to be passively sensitised with an IgE isotype of choice.

RBL-2H3 cell culture was maintained in Eagles minimum essential medium, containing 10% Foetal Bovine Serum (FBS) (heat activated), 100 U/ml Penicillin, 100 mg/ml Streptomycin and 2 mM L-glutamine.

Previous research indicates that RBL-2H3 cells derive their GPI-PLD from the culture serum (data not shown). Therefore, it follows that inactivation of this external source of GPI-PLD would deprive the cells of any further enzyme.

Inactivation of GPI-PLD activity in foetal bovine serum was achieved according to the method of Kung et al (Biochimica et Biophysica Acta, 1357:329-338, 1997). Briefly, FCS was adjusted to pH 11 using concentrated hydrochloric acid, and incubated for 1 hour at 37°C using. After this time, the pH was adjusted to 7.4, and GPI-PLD activity was determined using an enzymatic assay (Davitz et al, J. Biol. Chem., 264:13760-13764, 1989). Results indicated that this alkaline incubation severely depleted GPI-PLD activity (data not shown).

To determine the effect of culture of RBL-2H3 cells in GPI-PLD inactive serum, the supplemented MEM was replaced with MEM in which the FBS had been inactivated. Although the cell appearance was not dramatically altered by the altered culture conditions, determination of GPI-PLD activity showed a dramatic reduction in activity.

GPI-PLD activity in cells cultured with GPI-PLD active/inactive FBS:

Active = 0.66 units GPI-PLD activity/mg of protein.

Inactive = 0.11 units GPI-PLD activity/mg of protein.

The effect of a reduced GPI-PLD activity on the cell's ability to respond to IgE cross-linking was determined as follows:

RBL-2H3 cells were grown to confluence, after which time the adherent cells were removed from the culture flask using a cell scraper. The cell density was determined, using a haemocytometer, and adjusted to 2 x 10^5 per ml. The cells were seeded at 1 ml per well in a 24 well culture plate and cultured for overnight at 37°C in a humidified 5% CO_2 incubator.

The overnight culture media was aspirated and replaced with fresh media containing Rat IgE anti-DNP 3mg/ml. After a 2 hour incubation period, the media was aspirated, and the cells were washed twice, with HEPES Buffered Saline. Cross-linking was achieved by the addition of 200 ml of DNP-Albumin at 100 ng/ml, and incubation for 2 hours. Mediator release was determined using a colorimetric assay to detect the presence of b-hexosaminidase and compared with the total cell b-hexosaminidase content (as determined by incubation with 200 ml 5% Triton X-100 detergent). (Yasuda et al, Int. Imunol., 7:251-258, 1995). As shown in the table below, the responsiveness to cross-linking was significantly reduced in those cells that were cultured in GPI-PLD inactive media.

Percentage release in IgE linking activity assay (compared with total):

Active GPI-PLD culture = 48.79%

Inactive GPI-PLD culture = 5.07%

Phosphorylation of GPI-PLD

The phosphorylation state of the GPI-PLD enzymes was determined using MALDI-TOF mass spectrometry as described by Yip & Hutchins (1992). Spectrums of tryptic digests of the proteins can be compared before and after treatment with calf intestinal alkaline phosphatase. The specific kinases responsible for phosphorylation of GPI-PLD can then be determined by incubation of the GPI-PLD tryptic fragments with ATP in the presence of various kinases. Motif analysis of the amino acid sequence of human GPI-PLD using the HGMP "motif" package has revealed the presence of numerous potential phosphorylation sites for the enzymes cAMP-dependent protein kinase A, protein kinase C and protein kinase ck2 (formerly known as casine kinase II). Of these sites we have found that the site at amino acids 689-692 is a key site which when phosphorylated, e.g. by PKA, inhibits GPI-PLD biological activity.

These enzymes may therefore be involved in the activation/inactivation of GPI-PLD. Intriguingly the activity of protein kinase ck2 has been shown to be modulated by IPGs (Alemany et al, 1990) and there is also indirect evidence suggesting that IPGs may act through protein kinase C, thus suggesting the possibility of feedback loops regulating the production of IPGs.

Discussion

GPI-PLD is a metalloenzyme with 5 and 10 atoms per molecule of calcium and zinc, respectively. It circulates in a complex with apolipoprotein A1. GPI-PLD is produced in the pancreas by both a and b-cells in the islets of Langerhans. It is also produced by a mouse

insulinoma cell line (bTC3), with GPI-PLD and insulin generally colocalised intracellularly. The enzyme was shown to be secreted in response to insulin secretagogues. Both isoenzymes of GPI-PLD also seem to be present in liver; a major part of the activity could be washed away from the tissue by extraction with detergent-free buffer (thus, likely to be the plasma enzyme). There is some suggestions that the liver, as well as the pancreas, may contribute to the serum pool of GPI-PLD as patients with liver disease have lower levels of active enzyme, which is correlated with the reduced albumin levels.

It has been shown that streptozotcin-induced diabetes mellitus in the rat reduced the basal content of insulin-sensitive IPG in isolated hepatocytes by about 60%. The authors conclude that insulin resistance in these rats is related to the impairment of IPG metabolism. It has also been shown that the mRNA for a GPI-PLD-like gene was over expressed in genetically obese (ob/ob) mice in comparison to lean litter mates. In the context of the invention described herein, this latter finding suggests that GPI-PLD levels are responsive to the obese/diabetic genotype.

References:

The references mentioned herein are all incorporated by reference in their entirety.

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Claims:

1. Glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for use in a method of medical treatment.

2. The GPI-PLD of claim 1, wherein the GPI-PLD is simultaneously or sequentially administered with apoliprotein A1.

3. A nucleic acid molecule encoding GPI-PLD for use in a method of medical treatment.

4. Use of GPI-PLD for the preparation of a medicament for the treatment of conditions that respond to GPI-PLD or which are characterised by reduced levels of GPI-PLD as compared to a normal patient.

5. Use of the presence or amount of GPI-PLD in a sample from a patient in the diagnosis of a condition that responds to GPI-PLD or which is characterised by reduced levels of GPI-PLD as compared to a normal patient.

6. The use of claim 5, wherein the presence or amount of GPI-PLD is determined by measuring one of its biological activities.

7. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of diabetes or diabetic complications.

8. The use of claim 7, wherein the diabetes is an insulin dependent form of diabetes.

9. The use of claim 7 or claim 8, wherein the diabetes is Type I or Type II diabetes.

10. The use of claim 7, wherein the complications of diabetes are due to insulin resistance.

11. The use of any one of claims 7 to 10, wherein the medicament further comprises insulin, a glucose sparing or insulin enhancing drug, an α-glucosidase inhibitor or drug to treat insulin sensitivity, a P and/or A-type inositolphosphoglycan (IPG) and/or an IPG antagonist.

12. Use of a nucleic acid molecule encoding GPI-PLD, in the preparation of a medicament for the treatment of diabetes or diabetic complications.

13. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of liver dysfunction or disorders involving pancreatectomies.

14. The use of claim 13, wherein the medicament further comprises apolipoprotein A1.

15. Use of a nucleic acid molecule encoding GPI-PLD in the preparation of a medicament for the treatment of liver dysfunction.

16. The use of any one of the preceding claims, wherein the liver dysfunction is characterised by reduced levels of apolipoprotein A1 and/or GPI PLD and/or apolipoprotein A1/GPI-PLD complex as compared to a normal patient.

17. Use of glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD.

18. The use of claim 17, wherein the condition is mediated by an endotoxin.

19. The use of claim 18, wherein the endotoxin is a glycolipid from a *Mycobacterium* or gram negative bacteria.

20. The use of any one of claims 17 to 19, wherein the condition is septic shock.

21. Use of a nucleic acid molecule encoding glycosylphosphatidyl inositol specific phospholipase D (GPI-PLD) for the preparation of a medicament for the treatment of a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD.

22. A cell line transformed with nucleic acid encoding GPI-PLD, and capable of expressing and secreting GPI-PLD, for use in a method of medical treatment.

23. The cell line of claim 22, wherein the cell line is capable of producing apolipoprotein A1.

24. The use of the cell line of claim 22 or claim 23, in the preparation of a medicament for treatment of diabetes or diabetic complications, liver dysfunction or disorders involving pancreatectomies, or a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock.

25. A pharmaceutical composition comprising a nucleic acid molecule encoding a GPI-PLD protein.

26. A pharmaceutical composition comprising a GPI-PLD

protein.

27. The composition of claim 22, further comprising apolipoprotein A1.

28. A method of diagnosing a condition selected from diabetes or diabetic complications, liver dysfunction or disorders involving pancreatectomies, a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising:

determining the amount of GPI-PLD or a product of GPI-PLD action in a sample from a patient and correlating the amount to standards to determine whether the patient has or is at risk from said condition.

29. The method of claim 28, which comprises the steps of:

(a) contacting a biological sample obtained from the patient with a solid support having immobilised thereon a binding agent having binding sites specific for GPI-PLD or the product of GPI-PLD action;

(b) contacting the solid support with one or more labelled developing agents capable of binding to unoccupied binding sites, bound GPI-PLD or product, or occupied binding sites; and,

(c) detecting the label of the developing agents specifically binding in step (b) to obtain a value representative of the amount of GPI-PLD or the product of GPI-PLD action in the sample.

30. The method of claim 28, wherein the amount of GPI-PLD in the sample is determined by measuring GPI-PLD activity.

31. The method of claim 28 or claim 29, wherein the

product of GPI-PLD action are acyl-IPGs or IPGs.

32. An isolated or substantially isolated GPI-PLD protein with an amino acid sequence as shown in Figure 3.

33. An isolated nucleic acid sequence encoding a GPI-PLD as shown in any one of Figures 4 to 6.

34. An isolated nucleic acid sequence encoding a GPI-PLD, with greater than 90% identity with any one of the nucleic acid sequences shown in Figures 4 to 6.

35. An expression vector comprising nucleic acid sequence encoding a GPI-PLD protein as shown in any one of Figures 4 to 6.

36. A variant GPI-PLD polypeptides differing in amino acid sequence in the region corresponding to amino acid residues 689-692 inclusive (RRFS) of human wild-type GPI-PLD.

37. The variant of claim 36 which comprises a substitution in the region corresponding to amino acids 689-692 of mature human wild-type GPI-PLD.

38. The variant of claim 37, wherein the substitution changes the serine residue at position 692 to an amino acid other than serine or threonine.

39. The variant of any one of claims 36 to 38 for use in a method of medical treatment.

40. An isolated nucleic acid molecule encoding the variant GPI-PLD polypeptide of any one of claims 36 to 38.

41. The nucleic acid of any one of claims 36 to 38 for use in a method of medical treatment.

42. An expression vector comprising the nucleic acid molecule of claim 41, operably linked to control sequences to direct its expression.

43. A host cell transformed with the nucleic acid molecule of claim 41 encoding a GPI-PLD variant polypeptide.

44. A method of producing a variant GPI-PLD polypeptides which comprises culturing the host cells of claim 43 so that the variant GPI-PLD polypeptide is expressed and isolating the polypeptide thus produced.

45. The method of claim 44 which comprises the further step of then formulating the variant GPI-PLD polypeptide in a composition.

Figure 1

Top: protein produced from cDNA clone A1
Mid: protein produced from Roche patent bovine liver sequence
Bot: protein produced from Roche patent human liver sequence

```
MSAFRLWPGLLIMLG-SLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDA
MSAFRFWSGLLMLLG-FLCPRSSPCGISTHIEIGHRALEFLHLQDGSINYKELLLRHQDA
MSAFRLWPGLLMIVMASLCHRGSSCGLSTHIEIGHRALEFLHLHNGHVNYKELLLEHQDA

YQAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFL
YQAGSVFPDSFYPSICERGQFHDVSESTHWTPFLNASVHYIRKNYPLPWDEDTEKLVAFL
YQAGTVFPDCFYPSLCKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFL

FGITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLA
FGITSHMVADVNWHSLGIENGFLRTMAAIDFHNSYPEAHPAGDFGGDVLSQFEFKFNYLS
FGITSHMVADVSWHSLGIEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLA

RRWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFL
RHWYVPAEDLLGIYRELYGRIVITKKAIVDCSYLQFLEMYAEMLAISKLYPTYSVKSPFL
RRWYVPVKDLLGIYEKLYGREVITENVIVDCSHIQFLEMYGEMLAVSKLYPSYSTKSPFL

VEQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPENPLFIACGGQQNHTQG
VEQFQEYFLGGLEDMAFWSTNIYHLTSTMLKNGTSNCNLPENP---LFITCGGQQNNTHG
VEQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCSLFENPENPLFIACGGQQNHTQG

SKMQKNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIG
SKVQKNGFHKNVTAALTKNIGKHINYTKRGVFFSVDSWTMDFLSFMYKSLERSIREMFIG
SKMQKNDFHRNLTSSLTENIDRNINYTERGVFFSVNSWTPDSMSFIYKALERNVRTMFIG

GSQLSQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRV
SSQP-LTHVSSPAASYYLSFPYTRLGWAMTSADLNQDGYGDLVVGAPGYSHPGRIHVGRV
GSQLSQKHISSPLASYFLSFPYARLGWAMTSADLNQDGYGDLVVGAPGYSRPGRIHIGRV

YLIYGNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGS
YLIYGNDLG-PRIDLDLDKEAHGILEGFQPSGRFGSAVAVLDFNVDGVPDLAVGAPSVGS
YLIYGNELGLPPVDLDLDKEAHGILEGFQPSGRFGSALAMLDFNMDGVPDLAVGAPSVGS

EQLTYKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPD-LVIGSP
EKLTYTGAVYVYFGSKQGQLSSSPNVTISCQDTYCNLGWTLLAADVDGDSEPDLFVIGSP
EQLTYKGAVYVYFGSKQGRMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPD-LVIGSP

FAPGGGKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLL
FAFGGGKQKGIVAAFYSGSSYSSREKLNVEAANWMVKGEEDFAWLGYSLHGVNVNNRTLL
FAPGGGKQKGIVAAFYSGPSLSNKEKLNVEAANWTVRGEEDFAWFGYSLHGVTVDNRTLL

LVGSPTWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGH
LAGSPTWKDTSSQGHLFRTRDEKQSPGRVYGYFPPICQSWFTISGDKAMGKLGTSLSSGH
LVGSPTWKNASRLGRLLHIRDEKKSLGRVYGYFPPNSQSWFTIVGDKAMGKLGTSLSSGH
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Figure 1 continued

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VLMNGTLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRF
VIVNGTRTQVLLVGAPTQDVVSKS-FLTMTLHQGGSTRMYELTPDSQPSLLSTFSGNRRF
VLMNGTLTQVLLVGAPTRDDVSKMAFLTMTLHQGGATRMYALTSDLQPPLLSTFSGDRRF

SRFGGVLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKC
SRFGGVLHLSDLDNDGLDEIIVAAPLRITDATAGLMGEEDGRVYVFNGKQITVGDVTGKC
SRFGGVLHLSDLDDDGVDEIIVAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKC

KSWITPCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVY
KSWVTPCPEEKAQYVLISPEAGSRFGSSVITVRSKEKNQVIIAAGRSSLGARLSGVLHIY
KSWMTPCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVY

SLGSD
RLGQD
SLGSD
```

Figure 2

Top: pancreatic-form cDNA sequence from GenBank database
mid: our sequence cloned from human liver cDNA library
bot: Roche patent pancreatic-form partial cDNA sequence

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     ------------------------------------------------------------
1    GTGACCTGCTTAGAGAGAAGCGGTGGGTCTGCACCTGGATTTTGGAGTCCCAGTGCTGCT   60
     ------------------------------------------------------------

1    ----------------------------------------------------ATGTCTGCT    9
61   GCAGCTCTGAGCATTCCCACGTCACCAGAGAAGCCGGTGGGCAATGAGAGCATGTCTGCT  120
     ------------------------------------------------------------

10   TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG   69
121  TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG  180
     ------------------------------------------------------------

70   TGTGGCCTTTCAACACACGTAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC  129
181  TGTGGCCTTTCAACACACATAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC  240
     ------------------------------------------------------------

130  AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA  189
241  AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA  300
     ------------------------------------------------------------

190  ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG  249
301  ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG  360
     ------------------------------------------------------------

250  TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC  309
361  TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC  420
     ------------------------------------------------------------

310  TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT  369
421  TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT  480
     ------------------------------------------------------------

370  TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG  429
481  TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG  540
     ------------------------------------------------------------

430  ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT  489
541  ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT  600
     ------------------------------------------------------------

490  GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT  549
601  GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT  660
     ------------------------------------------------------------

550  GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC  609
661  GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC  720
     ------------------------------------------------------------
```

Figure 2 continued

```
610  GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA  669
721  GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA  780
     ------------------------------------------------------------

670  GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC  729
781  GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC  840
     ------------------------------------------------------------

730  CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT  789
841  CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT  900
     ------------------------------------------------------------

790  CTAACAATCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG  849
901  CTAACAAGCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG  960
     ------------------------------------------------------------

850  TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT  909
961  TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT 1020
     ------------------------------------------------------------

910  TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT  969
1021 TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT 1080
     ------------------------------------------------------------

970  GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC 1029
1081 GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC 1140
     ------------------------------------------------------------

1030 AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG 1089
1141 AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG 1200
     ------------------------------------------------------------

1090 CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG 1149
1201 CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG 1260
     ------------------------------------------------------------

1150 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1209
1261 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1320
     ------------------------------------------------------------

1210 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1269
1321 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1380
     ------------------------------------------------------------

1270 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1329
1381 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1440
     ------------------------------------------------------------

1330 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1389
1441 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1500
     ------------------------------------------------------------

1390 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1449
1501 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1560
     ------------------------------------------------------------
```

Figure 2 continued

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1450 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1509
1561 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1620
     ------------------------------------------------------------

1510 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1569
1621 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1680

     ------------------------------------------------------------

1570 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1629
1681 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1740
     ------------------------------------------------------------

1630 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC 1689
1741 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC 1800
1    -------------------------CTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC   35

1690 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 1749
1801 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 1860
36   GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC   95

1750 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG 1809
1861 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG 1920
96   CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG  155

1810 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1869
1921 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1980
156  AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG  215

1870 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 1929
1981 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 2040
216  GTGTATGGCTACTTCC-ACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA  275

1930 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 1989
2041 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 2100
276  ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGTCACGTACTGATGAATGGGACTCTGAAA  335

1990 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 2049
2101 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 2160
336  CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC  395

2050 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 2109
2161 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 2220
396  GTGACCCTACACCAAGGCGGAGCCACTCGCGTGTACGCACTCATATCTGACGCGCAGCCT  455

2110 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 2169
2221 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 2280
456  CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC  515

2170 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 2229
2281 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 2340
516  TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA  575
```

Figure 2 continued

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2230 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 2289
2341 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 2400
576  GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC  635

2290 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 2349
2401 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 2460
636  AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA  695


2350 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 2409
2461 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 2520
696  GAAGAAAAGGCGCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC  755

2410 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 2469
2521 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 2580
756  CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT  815

2470 TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT 2529
2581 TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT 2640
816  TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT  875

2530 CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT 2589
2641 CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT 2700
876  CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT  935

2590 TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC 2649
2701 TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC 2760
936  TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC  995


2650 CTGGGA---------------------------------------------------- 2655
2761 CTGGGA---------------------------------------------------- 2766
996  CTGGGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCA 1055

2656 ----------------------------GTAGAGAGACACACTAACAGCCACACCCTCTG 2687
2767 ----------------------------GTAGAGAGACACACTAACAGCCACACCCTCTG 2798
1056 GAAGGGAATTGTGGCTGCGTTTTATTGAGTAGAGAGACACACTAACAGCCACACCCTCTG 1115

2688 GAAATCTGATACAGTAAATATATGACTGCACCAGAAATATGTGAAATAGCAGACATTCTG 2747
2799 GAAATCTGATACAGTAAATATATGACTGCACCAG-------------------------- 2833
1116 GAAATCTGATACAGTAAATATATGACTACACCAGAAATATGTGAAATAGCAGACATTCTG 1175

2748 CTTACTCATGTCTCCTTCCACAGTTTACTTCCTCGCTCCCTTTGCATCTAAACCTTTCTT 2807
     ------------------------------------------------------------
1176 CTTACTCATGTCTCCTTCCACAGTTTACTTCCTCGCTCCCTTTGCATCTAAACCTTTCTT 1235

2808 CTTTCCCAACTTATTGCCTGTAGTCAGACCTGCTGTACAACCTATTTCCTCTTCCTCTTG 2867
     ------------------------------------------------------------
1236 CTTTCCCAACTTATTGCCTGTAGTC---------------------------------- 1261

2868 AATGTCTTTCCAGTGGCTGGAAAGGTCCCTCTGTGGTTATCTGTTAGAACAGTCTCTGTA 2927
     ------------------------------------------------------------
     ------------------------------------------------------------
```

Figure 2 continued

```
2928 CACAATTCCTCCTAAAAACATCCTTTTTTAAAAAAAGAATTGTTCAGCCATAAAGAAAGA 2987
     ------------------------------------------------------------
     ------------------------------------------------------------

2988 ACAAGATCATGCCCTTTGCAGGGACATGGATGGAGCTGGAGGCCATTATCCTTCATAAAC 3047
     ------------------------------------------------------------
     ------------------------------------------------------------

3048 TATTGCAGGAACAGAAAACCAAACACTCCATATTCTCACTTGTAAGTGGGAGCTAAGTGA 3107
     ------------------------------------------------------------
     ------------------------------------------------------------

3108 GAACACGTGGACACATAGAGGGAAACAACACACACTGGGGCCTATGAGAGGGCGGAAGGT 3167
     ------------------------------------------------------------
     ------------------------------------------------------------

3168 GGGAGGAGGGAGAGATCAGGAAAAATAACTAATGGATACTTAGGGTGATGAAATAATCTG 3227
     ------------------------------------------------------------
     ------------------------------------------------------------

3228 TGTAACAAACCCCCATGACACACCTTTATGTATGTAACAAACCAGCACTTCCTGCGCATG 3287
     ------------------------------------------------------------
     ------------------------------------------------------------

3288 TACCCCTGAACTTAAAAGTTAAAAAAAAGTTGAACTTAAAAATAACAGATTGGCCCATGC 3347
     ------------------------------------------------------------
     ------------------------------------------------------------

3348 CAATCAAAGTATAATAGAAAGCATAGTATAC 3378
     -------------------------------
     -------------------------------
```

Figure 3

cDNA clone d3

MILLFQDSMSFIYKALERNIRTMFIGGSQLSQKHVSSPLASYFLSFPYARLGWAMTSADL
NQDGHGDLVVGAPGYSRPGHIHIGRVYLIYGNDLGLPPVDLDLDKEAHRILEGFQPSGRF
GSALAVLDFNVDGVPDLAVGAPSVGSEQLTKGAVYVYFGSKQGGMSSSPNITISCQDIYC
NLGWTLLAADVNGDSEPDLVIGSPFAPGGGKQKGIVAAFYSGPSLSDKEKLNVEAANWTV
RGEEDFSWFGYSLHGVTVDNRTLLLVGSPTWKNASRLGHLLHIRDEKKSLGRVYGYFPPN
GQSWFTISGDKAMGKLGTSLSSGHVLMNGTLKQVLLVGAPTYDDVSKVAFLTVTLHQGGA
TRMYALISDAQPLLLSTFSGDRRFSRFGGVLHLSDLDDDGLDEIIMAAPLRIADVTSGLI
GGEDGRVYVYNGKETTLGDMTGKCKSWITPCPEEKAQYVLISPEASSRFGSSLITVRSKA
KNQVVIAAGRSSLGARLSGALHVYSLGSD

cDNA clone b2

MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY
QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF
GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR
RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV
EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ
KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL
SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY
GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT
YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG
GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP
TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG
TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG
VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT
PCPEEKVSEKKKKKK

cDNA clone a1

MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY
QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF
GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR
RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV
EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ
KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL
SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY
GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT
YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG
GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP
TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG
TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG
VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT
PCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVYSLGSD

Figure 4

2832 bp: 690 a 688 c 735 g 719 t

```
   1 gtgacctgct tagagagaag cggtgggtct gcacctggat tttggagtcc cagtgctgct
  61 gcagctctga gcattcccac gtcaccagag aagccggtgg gcaatgagag catgtctgct
 121 ttcaggttgt ggcctggcct gctgatcatg ttgggttctc tctgccatag aggttcaccg
 181 tgtggccttt caacacacat agaaatagga cacagagctc tggagtttct tcagcttcac
 241 aatgggcgtg ttaactacag agagctgtta ctagaacacc aggatgcgta tcaggctgga
 301 atcgtgtttc ctgattgttt ttaccctagc atctgcaaag gaggaaaatt ccatgatgtg
 361 tctgagagca ctcactggac tccgtttctt aatgcaagcg ttcattatat ccgagagaac
 421 tatccccttc cctgggagaa ggacacagag aaactggtag ctttcttgtt tggaattact
 481 tctcacatgg cggcagatgt cagctggcat agtctgggcc ttgaacaagg attccttagg
 541 accatgggag ctattgattt tcacggctcc tattcagagg ctcattcggc tggtgatttt
 601 ggaggagatg tgttgagcca gtttgaattt aattttaatt accttgcacg acgctggtat
 661 gtgccagtca aagatctact gggaatttat gagaaactgt atggtcgaaa agtcatcacc
 721 gaaaatgtaa tcgttgattg ttcacatatc cagttcttag aaatgtatgg tgagatgcta
 781 gctgtttcca agttatatcc cacttactct acaaagtccc cgtttttggt ggaacaattc
 841 caagagtatt ttcttggagg actggatgat atggcatttt ggtccactaa tatttaccat
 901 ctaacaagct tcatgttgga gaatgggacc agtgactgca acctgcctga gaaccctctg
 961 ttcattgcat gtggcggcca gcaaaaccac acccagggct caaaaatgca gaaaaatgat
1021 tttcacagaa atttgactac atccctaact gaaagtgttg acaggaatat aaactatact
1081 gaaagaggag tgttctttag tgtaaattcc tggaccccgg attccatgtc ctttatctac
1141 aaggctttgg aaaggaacat aaggacaatg ttcataggtg gctctcagtt gtcacaaaag
1201 cacgtctcca gccccttagc atcttacttc ttgtcatttc cttatgcgag gcttggctgg
1261 gcaatgacct cagctgacct caaccaggat gggcacggtg acctcgtggt gggcgcacca
1321 ggctacagcc gccccggcca catccacatc gggcgcgtgt acctcatcta cggcaatgac
1381 ctgggcctgc cacctgttga cctggacctg gacaaggagg cccacaggat ccttgaaggc
1441 ttccagccct caggtcggtt tggctcggcc ttggctgtgt tggactttaa cgtggacggc
1501 gtgcctgacc tggccgtggg agctccctcg gtgggctccg agcagctcac ctacaaaggt
1561 gccgtgtatg tctactttgg ttccaaacaa ggaggaatgt cttcttcccc taacatcacc
1621 atttcttgcc aggacatcta ctgtaacttg ggctggactc tcttggctgc agatgtgaat
1681 ggagacagtg aacccgatct ggtcatcggc tccccttttg caccaggtgg agggaagcag
1741 aagggaattg tggctgcgtt ttattctggc cccagcctga gcgacaaaga aaaactgaac
1801 gtggaggcag ccaactggac ggtgagaggc gaggaagact tctcctggtt tggatattcc
1861 cttcacggtg tcactgtgga caacagaacc ttgctgttgg ttgggagccc gacctggaag
1921 aatgccagca ggctgggcca tttgttacac atccgagatg agaaaaagag ccttgggagg
1981 gtgtatggct acttcccacc aaacggccaa agctggttta ccatttctgg agacaaggca
2041 atggggaaac tgggtacttc cctttccagt ggccacgtac tgatgaatgg gactctgaaa
2101 caagtgctgc tggttggagc ccctacgtac gatgacgtgt ctaaggtggc attcctgacc
2161 gtgaccctac accaaggcgg agccactcgc atgtacgcac tcatatctga cgcgcagcct
2221 ctgctgctca gcaccttcag cggagaccgc cgcttctccc gatttggtgg cgttctgcac
2281 ttgagtgacc tggatgatga tggcttagat gaaatcatca tggcagcccc cctgaggata
2341 gcagatgtaa cctctggact gattggggga gaagacggcc gagtatatgt atataatggc
2401 aaagagacca cccttggtga catgactggc aaatgcaaat catggataac tccatgtcca
2461 gaagaaaagg cccaatatgt attgatttct cctgaagcca gctcaaggtt tgggagctcc
2521 ctcatcaccg tgaggtccaa ggcaaagaac caagtcgtca ttgctgctgg aaggagttct
2581 ttgggagccc gactctccgg ggcacttcac gtctatagcc ttggctcaga ttgaagattt
2641 cactgcattt ccccactctg cccacctctc tcatgctgaa tcacatccat ggtgagcatt
2701 ttgatggaca aagtggcaca tccagtggag cggtggtaga tcctgataga catggggctc
2761 ctgggagtag agagacacac taacagccac accctctgga aatctgatac agtaaatata
2821 tgactgcacc ag
```

Figure 5

2472 bp: 617 a 588 c 639 g 628 t

```
   1 gtctgcacct ggattttgga gtcccagtgc tgctgcagct ctgagcattc ccacgtcacc
  61 agagaagccg gtgggcaatg agagcatgtc tgctttcagg ttgtggcctg gcctgctgat
 121 catgttgggt tctctctgcc atagaggttc accgtgtggc ctttcaacac acatagaaat
 181 aggacacaga gctctggagt ttcttcagct tcacaatggg cgtgttaact acagagagct
 241 gttactagaa caccaggatg cgtatcaggc tggaatcgtg tttcctgatt gtttttaccc
 301 tagcatctgc aaaggaggaa aattccatga tgtgtctgag agcactcact ggactccgtt
 361 tcttaatgca agcgttcatt atatccgaga gaactatccc ctttccctggg agaaggacac
 421 agagaaactg gtagctttct tgtttggaat tacttctcac atggcggcag atgtcagctg
 481 gcatagtctg ggccttgaac aaggattcct taggaccatg ggagctattg attttcacgg
 541 ctcctattca gaggctcatt cggctggtga ttttggagga gatgtgttga gccagtttga
 601 atttaatttt aattaccttg cacgacgctg gtatgtgcca gtcaaagatc tactgggaat
 661 ttatgagaaa ctgtatggtc gaaaagtcat caccgaaaat gtaatcgttg attgttcaca
 721 tatccagttc ttagaaatgt atggtgagat gctagctgtt tccaagttat atcccactta
 781 ctctacaaag tccccgtttt tggtggaaca attccaagag tattttcttg gaggactgga
 841 tgatatggca ttttggtcca ctaatattta ccatctaaca agcttcatgt tggagaatgg
 901 gaccagtgac tgcaacctgc ctgagaaccc tctgttcatt gcatgtggcg gccagcaaaa
 961 ccacacccag ggctcaaaaa tgcagaaaaa tgattttcac agaaatttga ctacatccct
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1081 ttcctggacc ccggattcca tgtcctttat ctacaaggct ttggaaagga acataaggac
1141 aatgttcata ggtggctctc agttgtcaca aaagcacgtc tccagcccct tagcatctta
1201 cttcttgtca tttccttatg cgaggcttgg ctgggcaatg acctcagctg acctcaacca
1261 ggatgggcac ggtgacctcg tggtgggcgc accaggctac agccgccccg gccacatcca
1321 catcgggcgc gtgtacctca tctacggcaa tgacctgggc ctgccacctg ttgacctgga
1381 cctggacaag gaggcccaca ggatccttga aggcttccag ccctcaggtc ggtttggctc
1441 ggccttggct gtgttggact ttaacgtgga cggcgtgcct gacctggccg tgggagctcc
1501 ctcggtgggc tccgagcagc tcacctacaa aggtgccgtg tatgtctact ttggttccaa
1561 acaaggagga atgtcttctt cccctaacat caccatttct tgccaggaca tctactgtaa
1621 cttgggctgg actctcttgg ctgcagatgt gaatggagac agtgaacccg atctggtcat
1681 cggctccct tttgcaccag gtggagggaa gcagaaggga attgtggctg cgttttattc
1741 tggccccagc ctgagcgaca aagaaaaact gaacgtggag gcagccaact ggacggtgag
1801 aggcgaggaa gacttctcct ggtttggata ttcccttcac ggtgtcactg tggacaacag
1861 aaccttgctg ttggttggga gcccgacctg gaagaatgcc agcaggctgg gccatttgtt
1921 acacatccga gatgagaaaa agagccttgg gagggtgtat ggctacttcc caccaaacgg
1981 ccaaagctgg tttaccattt ctggagacaa ggcaatgggg aaactgggta cttccctttc
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2281 agatgaaatc atcatggcag ccccctgag gatagcagat gtaacctctg gactgattgg
2341 gggagaagac ggccgagtat atgtatataa tggcaaagag accacccttg gtgacatgac
2401 tggcaaatgc aaatcatgga taactccatg tccagaagaa aaggtaagtg aaaaaaaaaa
2461 aaaaaaaaaa aa
```

Figure 6

1942 bp: 455 a 496 c 502 g 489 t

```
   1 gggctgtaac tctgccatcc ctcagcataa tttgggggta tgatttcact atcctaattg
  61 cctgtcctaa gtgatcttac ttgctgatag gacctaatgt tttattttat tgtttagcac
 121 ttctaaaaac tcatttcctt tacacaagtc caatactttg gacaggaaac agtagctttg
 181 ttgattatgc tacgtgtctt tactgtctat aatgattctt ttatttcagg attccatgtc
 241 ctttatctac aaggctttgg aaaggaacat aaggacaatg ttcataggtg gctctcagtt
 301 gtcacaaaag cacgtctcca gcccccttagc atcttacttc ttgtcatttc cttatgcgag
 361 gcttggctgg gcaatgacct cagctgacct caaccaggat gggcacggtg acctcgtggt
 421 gggcgcacca ggctacagcc gccccggcca catccacatc gggcgcgtgt acctcatcta
 481 cggcaatgac ctgggcctgc cacctgttga cctggacctg gacaaggagg cccacaggat
 541 ccttgaaggc ttccagccct caggtcggtt tggctcggcc ttggctgtgt tggactttaa
 601 cgtggacggc gtgcctgacc tggccgtggg agctccctcg gtgggctccg agcagctcac
 661 ctacaaaggt gccgtgtatg tctactttgg ttccaaacaa ggaggaatgt cttcttcccc
 721 taacatcacc atttcttgcc aggacatcta ctgtaacttg ggctggactc tcttggctgc
 781 agatgtgaat ggagacagtg aacccgatct ggtcatcggc tccccttttg caccaggtgg
 841 agggaagcag aagggaattg tggctgcgtt ttattctggc cccagcctga gcgacaaaga
 901 aaaactgaac gtggaggcag ccaactggac ggtgagaggc gaggaagact tctcctggtt
 961 tggatattcc cttcacggtg tcactgtgga caacagaacc ttgctgttgg ttgggagccc
1021 gacctggaag aatgccagca ggctgggcca tttgttacac atccgagatg agaaaaagag
1081 ccttgggagg gtgtatggct acttcccacc aaacggccaa agctggttta ccatttctgg
1141 agacaaggca atggggaaac tgggtacttc cctttccagt ggccacgtac tgatgaatgg
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1381 cgttctgcac ttgagtgacc tggatgatga tggcttagat gaaatcatca tggcagcccc
1441 cctgaggata gcagatgtaa cctctggact gattggggga gaagacggcc gagtatatgt
1501 atataatggc aaagagacca cccttggtga catgactggc aaatgcaaat catggataac
1561 tccatgtcca gaagaaaagg cccaatatgt attgatttct cctgaagcca gctcaaggtt
1621 tgggagctcc ctcatcaccg tgaggtccaa ggcaaagaac caagtcgtca ttgctgctgg
1681 aaggagttct ttgggagccc gactctccgg ggcacttcac gtctatagcc ttggctcaga
1741 ttgaagattt cactgcattt ccccactctg cccacctctc tcatgctgaa tcacatccat
1801 ggtgagcatt ttgatggaca aagtggcaca tccagtggag cggtggtaga tcctgataga
1861 catggggctc ctgggagtag agagacacac taacagccac accctctgga aatctgatac
1921 agtaaatata tgactgcacc ag
```

Figure 7

```
database    MSAFRLWPGLLIMLGSLCHRGSPCGLSTHVEIGHRALEFLQLHNGRVNYRELLLEHQDAY   60
d3          ------------------------------------------------------------
b2          MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY   60
a1          MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY   60

database    QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF  120
d3          ------------------------------------------------------------
b2          QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF  120
a1          QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF  120

database    GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR  180
d3          ------------------------------------------------------------
b2          GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR  180
a1          GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR  180

database    RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV  240
d3          ------------------------------------------------------------
b2          RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV  240
a1          RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV  240

database    EQFQEYFLGGLDDMAFWSTNIYHLTIFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ  300
d3          ------------------------------------------------------------
b2          EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ  300
a1          EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ  300

database    KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL  360
d3          ------------------------------MILLFQDSMSFIYKALERNIRTMFIGGSQL   30
b2          KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL  360
a1          KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL  360

database    SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY  420
d3          SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY   90
b2          SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY  420
a1          SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY  420

database    GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  480
d3          GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  150
b2          GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  480
a1          GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  480

database    YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  540
d3          YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  210
b2          YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  540
a1          YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  540
```

Figure 7 continued

```
database    GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  600
d3          GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  270
b2          GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  600
a1          GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  600

database    TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  660
d3          TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  330
b2          TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  660
a1          TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  660

database    TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  720
d3          TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  390
b2          TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  720
a1          TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  720

database    VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT  780
d3          VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT  450
b2          VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT  780
a1          VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT  780

database    PCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVYSLGSD  840
d3          PCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVYSLGSD  510
b2          PCPEEKVSEKKKKKK-----------------------------------------------  795
a1          PCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVYSLGSD  840

Database    840 aa
d3          510 aa
b2          795 aa
a1          840 aa
```

Figure 8

1: pancreatic-form: cDNA sequence from GenBank database (L11702)
2: cDNA clone A1
3: cDNA clone B2
4: cDNA clone D3

```
     ------------------------------------------------------------
1    GTGACCTGCTTAGAGAGAAGCGGTGGGTCTGCACCTGGATTTTGGAGTCCCAGTGCTGCT    60
1    --------------------------GTCTGCACCTGGATTTTGGAGTCCCAGTGCTGCT    34
     ------------------------------------------------------------

1    ----------------------------------------------------ATGTCTGCT     9
61   GCAGCTCTGAGCATTCCCACGTCACCAGAGAAGCCGGTGGGCAATGAGAGCATGTCTGCT   120
35   GCAGCTCTGAGCATTCCCACGTCACCAGAGAAGCCGGTGGGCAATGAGAGCATGTCTGCT    94
     ------------------------------------------------------------

10   TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG    69
121  TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG   180
95   TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG   154
     ------------------------------------------------------------

70   TGTGGCCTTTCAACACACGTAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC   129
181  TGTGGCCTTTCAACACACATAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC   240
155  TGTGGCCTTTCAACACACATAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC   214
     ------------------------------------------------------------

130  AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA   189
241  AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA   300
215  AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA   274
     ------------------------------------------------------------

190  ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG   249
301  ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG   360
275  ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG   334
     ------------------------------------------------------------

250  TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC   309
361  TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC   420
335  TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC   394
     ------------------------------------------------------------

310  TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT   369
421  TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT   480
395  TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT   454
     ------------------------------------------------------------

370  TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG   429
481  TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG   540
541  TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG   514
     ------------------------------------------------------------
```

Figure 8 continued

```
430  ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT   489
541  ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT   600
515  ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT   574
     ------------------------------------------------------------

490  GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT   549
601  GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT   660
575  GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT   634
     ------------------------------------------------------------

550  GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC   609
661  GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC   720
635  GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC   694
     ------------------------------------------------------------

610  GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA   669
721  GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA   780
695  GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA   754
     ------------------------------------------------------------

670  GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC   729
781  GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC   840
755  GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC   814
     ------------------------------------------------------------

730  CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT   789
841  CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT   900
815  CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT   874
     ----------------------------------------------------GGGCTGTAAC    10

790  CTAACAATCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG   849
901  CTAACAAGCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG   960
875  CTAACAAGCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG   934
11   TCTGCCATCCCTCAGCATAATTTGGGGGTATGATTTCACTATCCTAATTGCCTGTCCTAA    70

850  TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT   909
961  TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT  1020
935  TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT   994
71   GTGATCTTACTTGCTGATAGGACCTAATGTTTTATTTTATTGTTTAGCACTTCTAAAAAC   130

910  TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT   969
1021 TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT  1080
995  TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT  1054
131  TCATTTCCTTTACACAAGTCCAATACTTTGGACAGGAAACAGTAGCTTTGTTGATTATGC   180

970  GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC  1029
1081 GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC  1140
1055 GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC  1114
181  TACGTGTCTTTACTGTCTATAATGATTCTTTTATTTCAGGATTCCATGTCCTTTATCTAC   240
```

Figure 8 continued

```
1030 AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG 1089
1141 AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG 1200
1115 AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG 1174
241  AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG  300

1090 CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG 1149
1201 CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG 1260
1175 CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG 1234
301  CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG  360

1150 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1209
1261 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1320
1235 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1294
361  GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA  420

1210 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1269
1321 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1380
1295 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1354
421  GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC  480

1270 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1329
1381 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1440
1355 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1414
481  CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC  540

1330 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1389
1441 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1500
1415 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1474
541  TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC  600

1390 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1449
1501 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1560
1475 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1534
601  GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT  660

1450 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1509
1561 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1620
1535 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1594
661  GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC  720

1510 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1569
1621 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1680

1595 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1654
721  ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT  780

1570 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1629
1681 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1740
1655 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1714
781  GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG  840
```

Figure 8 continued

```
1630 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC  1689
1741 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC  1800
1715 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC  1774
841  AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC   900

1690 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC  1749
1801 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC  1860
1775 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC  1834
901  GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC   960

1750 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG  1809
1861 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG  1920
1835 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG  1894
961  CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG  1020

1810 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG  1869
1921 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG  1980
1895 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG  1954
1021 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG  1080

1870 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA  1929
1981 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA  2040
1955 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA  2014
1081 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA  1140

1930 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA  1989
2041 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA  2100
2015 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA  2074
1141 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA  1200

1990 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC  2049
2101 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC  2160
2075 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC  2134
1201 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC  1260

2050 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT  2109
2161 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT  2220
2135 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT  2194
1261 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT  1320

2110 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC  2169
2221 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC  2280
2195 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC  2254
1321 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC  1380

2170 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA  2229
2281 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA  2340
2255 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA  2314
1381 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA  1440
```

Figure 8 continued

```
2230 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC  2289
2341 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC  2400
2315 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC  2374
1441 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC  1500

2290 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA  2349
2401 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA  2460
2375 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA  2434
1501 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA  1560

2350 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC  2409
2461 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC  2520
2435 GAAGAAAAGGTAAGTGAAAAAAAAAAAAAAAAAAAAAAA----------------------  2472
1561 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC  1620

2410 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT  2469
2521 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT  2580
     ------------------------------------------------------------
1621 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT  1680

2470 TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT  2529
2581 TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT  2640
     ------------------------------------------------------------
1681 TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT  1740

2530 CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT  2589
2641 CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT  2700
     ------------------------------------------------------------
1741 CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT  1800

2590 TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC  2649
2701 TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC  2760
     ------------------------------------------------------------
1801 TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC  1860

2650 CTGGGAGTAGAGAGACACACTAACAGCCACACCCTCTGGAAATCTGATACAGTAAATATA  2709
2761 CTGGGAGTAGAGAGACACACTAACAGCCACACCCTCTGGAAATCTGATACAGTAAATATA  2820
     ------------------------------------------------------------
1861 CTGGGAGTAGAGAGACACACTAACAGCCACACCCTCTGGAAATCTGATACAGTAAATATA  1920

2710 TGACTGCACCAGAAATATGTGAAATAGCAGACATTCTGCTTACTCATGTCTCCTTCCACA  2769
2821 TGACTGCACCAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  2880
     ------------------------------------
1921 TGACTGCACCAGAAAAAAAAAAAAAAAAAA                                1952
```

Figure 8 continued

```
2770 GTTTACTTCCTCGCTCCCTTTGCATCTAAACCTTTCTTCTTTCCCAACTTATTGCCTGTA  2829
2881 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA----------------------  2915
     ------------------------------------------------------------
     ------------------------------------------------------------

2830 GTCAGACCTGCTGTACAACCTATTTCCTCTTCCTCTTGAATGTCTTTCCAGTGGCTGGAA  2889
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------

2890 AGGTCCCTCTGTGGTTATCTGTTAGAACAGTCTCTGTACACAATTCCTCCTAAAAACATC  2949
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------

2950 CTTTTTTAAAAAAAGAATTGTTCAGCCATAAAGAAAGAACAAGATCATGCCCTTTGCAGG  3009
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------

3010 GACATGGATGGAGCTGGAGGCCATTATCCTTCATAAACTATTGCAGGAACAGAAAACCAA  3069
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------

3070 ACACTCCATATTCTCACTTGTAAGTGGGAGCTAAGTGAGAACACGTGGACACATAGAGGG  3129
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------

3130 AAACAACACACACTGGGGCCTATGAGAGGGCGGAAGGTGGGAGGAGGGAGAGATCAGGAA  3189
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------

3190 AAATAACTAATGGATACTTAGGGTGATGAAATAATCTGTGTAACAAACCCCCATGACACA  3249
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------

3250 CCTTTATGTATGTAACAAACCAGCACTTCCTGCGCATGTACCCCTGAACTTAAAAGTTAA  3309
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------
```

Figure 8 continued

```
3310 AAAAAAGTTGAACTTAAAAATAACAGATTGGCCCATGCCAATCAAAGTATAATAGAAAGC  3369
     ------------------------------------------------------------
     ------------------------------------------------------------
     ------------------------------------------------------------


3370 ATAGTATAC  3378
     ---------
     ---------
     ---------
```